

nature

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Believing in OSTP's merits

PRESIDENT Carter last month sent Congress a proposal to reorganise the constellation of offices and advisory units which together constitute his immediate White House and Executive Office staff. The proposal is the first of many promised moves to streamline and prune the federal bureaucracy, a pledge which figured prominently in Mr Carter's campaign rhetoric. For the fledgling Office of Science and Technology Policy (OSTP), the most important outcome of the proposed reorganisation is that it will be kept in business.

That fact alone signifies that OSTP has made an impression in the Carter administration during its short lifetime, for it is widely acknowledged that the people who planned the reorganisation were initially disposed to recommend that the office be disbanded. According to Dr Frank Press, Carter's science adviser and director of OSTP, the reorganisers approached the office as disbelievers, but went away as believers in OSTP's merits.

One reason for that belief is that Press has established a close working relationship with other White House units and he seems to enjoy easy access to the President, a fact which adds considerably to his standing in the White House power structure. He attends Cabinet meetings and daily meetings of Carter's senior staff, and OSTP is always represented during budget negotiations between agencies which have responsibility for R & D and the Office of Management and Budget. In addition, OSTP has been called upon to undertake a raft of studies ranging from a review of dam safety regulations to a survey of basic research policies in mission oriented agencies. Clearly, OSTP has established a solid niche.

Nevertheless, the reorganisation plan does not leave OSTP entirely unscathed. It will remove a number of functions specifically consigned to OSTP in the legislation which established the office last year, and it will result in a reduction of OSTP's staff level from the congressionally authorised 32 positions to 22. The net effect of the pruning will be to preserve and perhaps even strengthen those provisions related to the day-to-day advice to the President and other White House units, while stripping away some responsibilities for analysing longer term issues.

Although those losses may seem like a small price to pay for retaining OSTP in the White House, they are cause for concern. In fact, there has recently been some grumbling from OSTP's chief sponsors on Capitol Hill,

notably Olin Teague, chairman of the House Committee on Science and Technology, and Senator Adlai Stevenson, chairman of the Senate Commerce Subcommittee on Science, Technology and Space. Both have sent letters to the chairmen of the House and Senate Government Operations Committees, which will be reviewing the reorganisation plan, expressing concern at the potential loss of some of OSTP's functions.

Specifically, the reorganisation plan would relieve OSTP of the responsibility for preparing two reports, a five-year outlook identifying problems and issues likely to require attention in the years ahead, and a series of annual reports reviewing developments in science and technology and assessing selected government programmes. Both those tasks will now be undertaken by the National Science Foundation, which is probably as well equipped as OSTP to write such reports, but studies carrying a White House imprimatur would carry much more weight.

The reorganisation plan would also do away with the President's Committee on Science and Technology (PCST), a top-level advisory committee similar to the former powerful President's Science Advisory Committee. PCST was established by Congress primarily to conduct a two-year review of federal science policies and to recommend organisational changes in the federal bureaucracy dealing with science and technology. At the end of PCST's review, Carter would have had the option of keeping the committee as a permanent advisory body.

The committee will be scrapped chiefly because a White House unit is already looking at government reorganisation—having a separate committee looking into the organisation of science agencies was deemed superfluous. Nevertheless, the committee's demise will remove a potentially important source of outside opinion particularly on longer term issues which lie beyond the day-to-day matters facing the White House.

When Congress created OSTP last year, it gave it the dual role of advising the President and other White House units on issues involving science and technology, and of providing long range analysis of broad questions concerned with science and social policy. The reorganisation seems to have strengthened the first role by ensuring that the Science Adviser will have a secure place in the upper echelons of the White House. But the second function has been downgraded. □



Science in the Himalayas

Peter D. Moore, recently in India, finds some scientific surprises in the mountains

ANYONE who travels in India soon grows accustomed to the unexpected. Even so, the little town of Dehra Dun, nestling in the foothills of the Himalayas ten hours' train journey north of Delhi, will surprise the most hardened traveller, for it occupies an influential position in Indian science. It serves as a centre for research, training and exploration in such industries as oil and natural gas and in forestry, and it is also the base for the Northern Region of the Botanical Survey of India.

The most unexpected of these is perhaps the oil industry, especially when one considers the distribution of oil and natural gas exploitation in India. At present there are two major sedimentary basins yielding oil (see accompanying map), located at the eastern and western extremities of the sub-continent. In the east is the Assam-Arakan basin, which is being exploited by both the Indian Oil and Natural Gas Commission (ONGC) and by Oil India Ltd, in which the Indian Government and the British Burma Oil Company hold equal shares. At present Oil India is producing 3.08×10^6 tons annually from Assam, representing more than a third of India's total oil production. Further development of this field could double its output. In the west of India, in Gujarat, is the Cambay Basin, which extends from the coast to the gas-bearing strata of Rajasthan and south into the Gulf of Cambay. This field was discovered in the late 1950s and since then the offshore fields of the Bombay High have also been brought into production.

Crude oil production has risen from 259,000 tons a year in 1951 to a present output of about 8×10^6 tons. Greatest expansion was achieved between 1963 and 1969. At the same time annual consumption of petroleum products has risen from 3.9×10^6 tons in 1951 to a present level of about 23×10^6 tons. India is thus producing only one third of her oil needs.

Oil consumption in India will undoubtedly continue to grow. Apart from her fuel requirements, the growth of the petrochemical and fertiliser industries will present an increasing demand. There is already talk of lining canals with polythene to prevent water losses, and even the possibility of covering whole fields. In fishing, clothing and domestic construction, particularly in the improvement of water supplies and sewerage, plastic

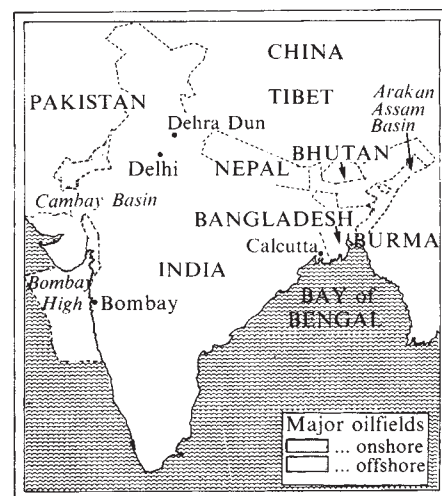
products will contribute to efficiency and to health. Historically, many apparently profound influences have been passively absorbed by India and have been swallowed by the inertia of her ageless culture, leaving her unchanged. Will plastics really alter the face of India? One wonders whether the Indian's concern for the welfare of bovines will actually result in a plastic bullock cart fitted with pneumatic tyres—a suggestion recently made by Dr S. Varadarajan, Chairman of Indian Petrochemicals Ltd.

Growing demand

On the fertiliser side, the current production of about 1.5×10^6 tons of nitrogen has placed a growing demand upon crude oil. Many plants consume sulphur-rich fuel oil, which can be imported relatively cheaply because of the air pollution resulting from SO_2 production on combustion and because of the strength of the ecological lobby in the industrialised nations. India, like so many developing nations, does not yet feel in a position to afford the clean air which the developed nations now vainly seek.

What India also cannot afford is that its oil import bill should continue to rise. Great emphasis is now placed, therefore, upon the closure of the gap between home production and consumption of petroleum products. Optimistic predictions abound in India at present. Prospects for the development of existing fields, particularly the offshore drilling in the Bombay High, are good. Proven reserves on land in India are estimated at 700×10^6 tons, of which 200×10^6 tons are considered recoverable, while the Bombay High is thought to contain at least another 800×10^6 tons. N. B. Prasad, chairman of ONGC, considers that production from these major fields can rise to about 20×10^6 tons a year within the next four years, thus narrowing very considerably the gap between production and demand in the country.

The political dream of an India self-supporting in her oil needs, however, still demands further exploration and development. This is where the Himalayan town of Dehra Dun comes into the picture. In 1963 an Institute of Petroleum Exploration was set up with the aid of money from the United Nations Development Programme. The apparently strange choice of site (almost equally distant from both of the major oil producing areas) can be



explained by the contemporary search for oil in the Himalaya foothills. In the fourteen years since the inception of this institute no reserves of a commercially viable nature have yet been discovered, thus leaving it stranded in a remote but scenic location which was once the summer playground of the British Raj.

Exploration and analytical research remain the prime functions of the Dehra Dun Institute. A comprehensive evaluation of the sedimentary basins of India is under way, no mean task given that it covers basin areas of 10^6 km^2 onshore and $320,000 \text{ km}^2$ offshore. The volume of chemical, geological and palynological data generated in the laboratories at Dehra Dun has necessitated the installation of an IBM 370/145 computer in the institute for rapid assessment of oil-bearing potential. Russian influence at the Institute has been and remains strong. The original UN representative at the establishment of the institute was a Russian and a team of scientists from the Soviet Union assists in research and analysis.

Forestry too

The Institute of Petroleum Exploration is not the only surprise awaiting the visitor to Dehra Dun. Just 6 km west of the town stands a most extraordinary and impressive building set in 445 ha of lawns and gardens. It houses the Indian Forest Research Institute. Built in 1929 and inaugurated by the then Viceroy of India, Lord Irwin, this institute is a monument to the economic importance of forestry in India. It began as the Imperial Forest School in 1884 and then expanded to encompass forest research. It now serves both a research and teaching function and houses a staff of 1,900.

As in many other countries, India has suffered badly from deforestation and the resultant soil erosion. This has reputedly come about particularly since Moghul times in north India. It is said

(usually by Hindus) that the Moham-medans did not possess the same respect for vegetation as their Hindu predecessors. It is certainly true that vast areas of the Himalayan foothills have been stripped of forest and now only fragments remain. This has come about both as a result of intensive grazing and also the use of wood for fuel.

The varied topography in the region of Dehra Dun provides an ideal setting for a Forestry Institute. The steep slopes of the Lesser Himalayas take one up out of the sub-tropical climate of the plains and the Siwalik Hills into the cool temperate climate of the foot-hill ridges. Broad features of forest zonation accompany these altitude changes. It is thus possible for students at the Forest Research Institute to gain experience in a variety of forest conditions.

Besides the practical aspects of silviculture, the institute offers training and research facilities in such fields as entomology, paper technology, wood physics, timber mechanics and genetics. The main buildings house a vast museum and herbarium. The institute has served an important function in the development of techniques suitable for the conditions found in India. For example, the use of bamboo as a raw material in the manufacture of paper was pioneered here, as was the use of sugar cane waste. Bamboo, the poor man's timber and the standard material for scaffolding in India, represents a jungle to the taxonomist. There are about a hundred species and they flower only once, immediately prior to death, so that by the time you can identify them it is too late. Techniques for identification on the basis of culm sheaths and young bud structure are currently being worked upon, since identification is essential for the further development of bamboo management methods.

Besides the obvious products of forestry, timber and pulp, the Indian forests are rich in species which are economically important as a source of minor products. For example, the oil from sandalwood is an important earner of foreign exchange for India. The production of santonin from certain *Artemisia* species is an area which has recently received attention. The survey of Indian plants for potentially useful species is thus of considerable importance. The Forest Research Institute deals with woody plants, but what of the others?

Botanical survey

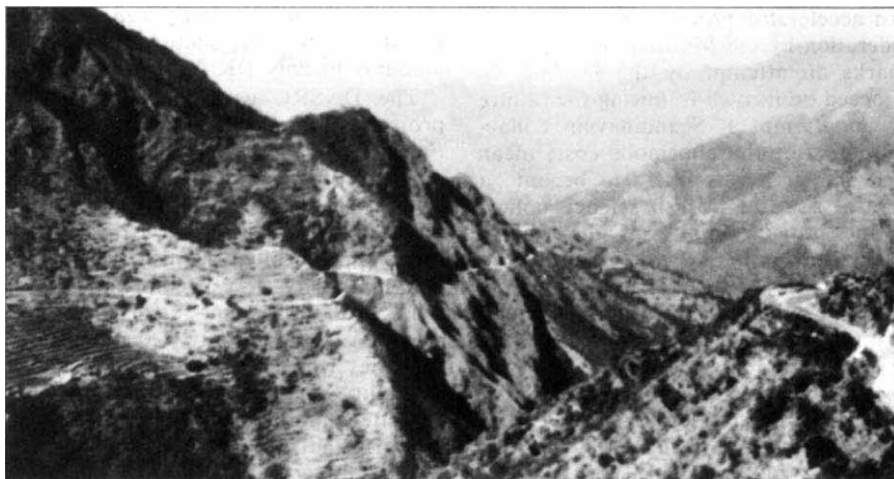
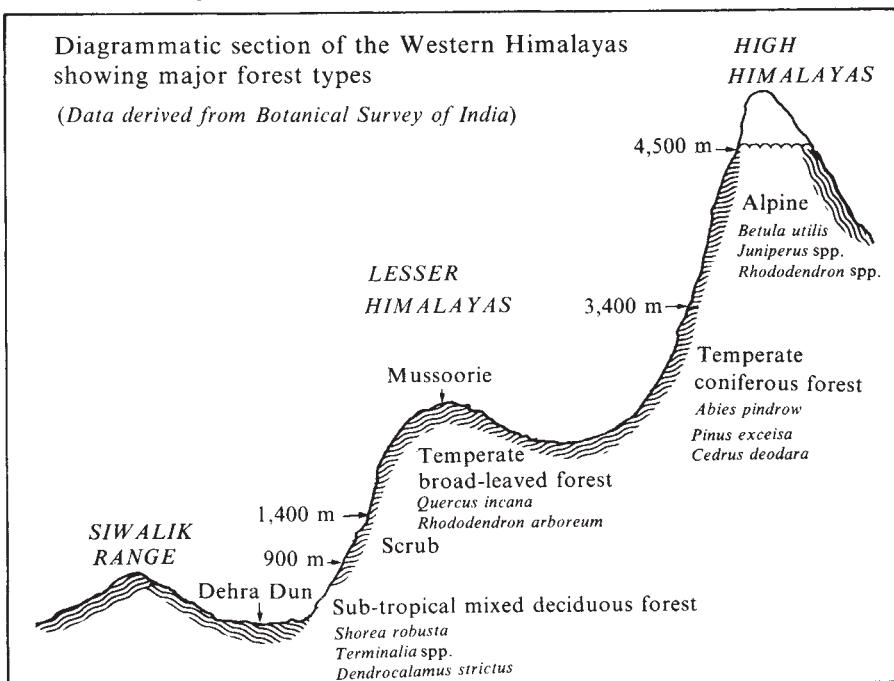
It comes as no surprise to find yet another institute tucked away in the suburbs of Dehra Dun, this time the headquarters of the Northern Circle of the Botanical Survey of India. This is a

government organisation which is centred on Calcutta and which functions through four Regional Circles. The emphasis is upon plants as a national resource (in an economic rather than an aesthetic sense), but the bulk of the work is purely taxonomic. At present the flora of India has still not been fully described and the high Himalayas represent a particularly underworked area.

Because a major aim of the survey is the discovery of plants with a commercial potential, especially those with medicinal properties, the development of a herbarium of dried specimens is not enough; plants must be kept alive. The establishment of a botanical garden is therefore a priority and, as many of the more interesting plants derive from high altitude, the garden must also be sited in the hills. The British developed an upland botanic garden at Mussoorie (about 2,200 m) but this has now been abandoned as a scientific venture. A new garden has been estab-

lished at Pauri (1,500 m) which will be used to maintain populations of high altitude plants and for experimentation. Such a venture may also prove to be valuable for the conservation of some rarer species, such as some of the orchids, and may also provide material for horticultural developments.

Perhaps the plant resources of the Himalayas will soon provide the basis for further commercial exploitation in the form of tourism. It still takes a considerable muscular effort to reach the Bhyundhar Valley (the 'Valley of Flowers') at 3,600 m but one can envisage changes coming about as India seeks more opportunities to earn foreign exchange. Although the Hindu pilgrims are prepared to trek on foot to their mountain shrines, the Western tourist will undoubtedly demand the development and despoilation of these remote areas. Conservatism, a right sense of priorities, or even sheer inertia may make India unwilling to pay the price. □



Cultivated terraces near Mussoorie in the Himalayas

EARTHQUAKES

Warning in time

Accounts of recent successful earthquake predictions in China have reached Western scientists. Colin Norman reports

THREE successful earthquake predictions were made in China last year, and warnings were issued in time to evacuate people from hazardous buildings in the threatened area. But there were also some failures. A number of false alarms were issued, and Chinese scientists failed to predict the devastating earthquake which levelled the city of T'angshan in July last year.

Those recent successes and failures were discussed for the first time by Chinese scientists who attended an international meeting on earthquake prediction sponsored by UNESCO (the United Nations Educational, Scientific and Cultural Organization) in Paris late last month. According to American scientists who attended the meeting, the Chinese were frank in their discussions and provided much previously unreported information on their methods.

Interest in China's earthquake prediction programme has been intense

ever since it became known in the West that Chinese scientists had accurately predicted a major earthquake which struck the city of Haicheng in February 1975. That prediction led to evacuation of the city and it is credited with saving many lives.

The latest successful predictions, according to Robert L. Wesson, a seismologist from the US Geological Survey who attended the UNESCO meeting, involved three earthquakes in 1976 of about magnitude 7 on the Richter Scale. They occurred in Yunnan Province on 29 May, in Szechuan Province on 16 August, and in the Szechuan-Yunnan border region on 7 November.

Wesson reported that in each case, Chinese scientists made long-term predictions that an earthquake would occur. Based on a variety of geophysical and magnetic anomalies, the long-term predictions were used as a basis for more intense monitoring in the threatened area, and for disaster planning. They were, however, not made public.

Public warnings that an earthquake was imminent were issued only on the appearance of foreshocks—minor tremors

which sometimes precede major quakes. The public warnings, according to Wesson, were accompanied by evacuation of people from potentially hazardous buildings. The appearance of foreshocks in the Haicheng area was also used as the trigger for issuing an earthquake warning there in 1975.

Major earthquakes frequently strike abruptly, however, with no foreshocks, and thus imminent hazard warnings may not be issued in time. That seems to have been the case with the T'angshan earthquake. Wesson said that the Chinese had made a medium-term prediction of a quake in the area which had underestimated the magnitude of the event and no imminent warning was issued. In the past, Chinese officials have been reluctant to discuss the T'angshan earthquake, which killed more than 600,000 people.

Wesson also said that the Chinese scientists at the UNESCO meeting admitted that several false alarms have been issued, although their success rate was not revealed. According to Dr Jack Savage, a geophysicist who has visited China, Chinese scientists have acknowledged issuing false alarms before, but they argue that they would prefer to issue warnings whenever necessary rather than risk the consequences of an unpredicted major quake. □

DENMARK

Big science in a small country

A new nuclear physics project is under consideration in Denmark. Sven Godtfredsen reports

THE fate of experimental nuclear physics at Denmark's Niels Bohr Institute is in the process of being decided. An accelerator project now under consideration by the Ministry of Education marks an attempt by the institute to proceed on its own following the failure of an attempt at Scandinavian collaboration. But the enormous costs mean that the matter is likely to become a central issue in Danish science policy.

The project, known as the Danish heavy ion project, was submitted to the Ministry of Education, by the Danish Council for Scientific Policy and Planning. It involves building a 20MV or 25MV tandem accelerator. The total cost of the project will include the cost of building the accelerator, estimated at Dkr115 million or Dkr86 million depending on the choice of terminal voltage, and the Dkr3 million increase

in grants to the institute for operating the accelerator (£1=Dkr10.4). Financially speaking, the project is breathtaking compared to expenditure on other natural science projects in Denmark. Grants from the Danish Natural Science Research Council (DNSRC) whose function is to finance and to some extent lead Danish research in the natural sciences, amounts to only Dkr25 million a year.

The DNSRC gave its view on the project last May in a report called *The Future of Nuclear Physics in Denmark*. Apart from examining the scientific content of the heavy ion project, the report reviews it in relation to broader issues—specifically, Danish participation in CERN, the attempts to establish collaboration in nuclear physics between the four Scandinavian countries (the NORDAC project), the possibility of collaboration between the institute and the heavy ion laboratory in Darmstadt, and of course, the particular financial aspects of nuclear physics in Denmark. But the question

really concerning the DNSRC is how long a small country like Denmark can run its own programme in such an expensive branch of natural science.

This question is not new. It was asked in 1970 when the Niels Bohr Institute needed to renew the 9MV tandem accelerator, and inspired nuclear physicists from the four Scandinavian countries to discuss the possibility of building a major heavy ion laboratory in the North. The idea was elaborated into the NORDAC project, which the DNSRC strongly recommended and which received financial assurances from the Danish government. Unfortunately the other Scandinavian countries were unable to back the project. Since then the institute has used the heavy ion tandem accelerator in Darmstadt for most of its nuclear physics experiments. But scientists at the institute feel that this approach weakens the contact between experimental and theoretical nuclear physics, traditionally the institute's strong feature.

There is little reason to believe that the NORDAC project is any more likely to happen now, so the Niels Bohr

Institute has decided to try to proceed on its own. Once again it has obtained full support from the DNSRC which argues that the proposed accelerator must be a national as well as an international asset. Current research at the institute needs an accelerator which provides a beam of very high energy heavy ions with good ionoptic properties and of very precise energy which can be varied rapidly. At present these requirements are only fulfilled by heavy ion tandem accelerators three of which

are currently under construction: at Oak Ridge, Tennessee, in the USA (25 MV), at Daresbury in Britain (20 MV, which can be upgraded to 30 MV) and at the Atomic Energy Research Institute in Japan (20 MV). The DNSRC believes that the kind of nuclear physics research which can be undertaken with these accelerators is so fruitful that more accelerators than these three instruments will be needed in the near future.

Both the DNSRC and the Danish

Council for Scientific Policy and Planning stress that the accelerator is required to continue the close interplay between theoretical and experimental work and "to sustain the status of the Niels Bohr Institute as a centre of excellence". Without the 25 MV or the 20 MV tandem they believe the institute will stagnate; with it they believe the institute will use its capabilities to provide results "with consequences for the world picture presented by modern physics". □

COMECON

● The thirty-second Session of the Comecon Permanent Commission for the Peaceful Uses of Atomic Energy which took place recently in Budapest discussed the long-term draft plan for fuel, energy and raw materials, cooperation in the use of radiation in industry, agriculture and environmental protection, the improvement of anti-radiation precautions and protection, and the disposal of radioactive waste. Also considered was the significance of cooperation in nuclear power for the overall coordinated plan for integrating the member countries' economies. To celebrate the thirtieth anniversary of Comecon, the Commission will publish, in 1979, a book entitled *The Peaceful Atom in the Countries of Socialism*.

Long-term energy and raw materials needs was also one of the themes of the recent meeting in Havana of the Permanent Commission on Power. In addition to discussing the long-term development of the unified power grids of the continental members of Comecon, special attention was paid to the particular needs of Cuba. A broad programme of new investments includes the construction of the first nuclear power station and the installation of transmission lines of higher tension than the present 220V. Cuba is already involved in a number of Comecon bilateral agreements for cooperation in nuclear and conventional energy with the USSR, East Germany, Hungary and Poland. According to J. L. Beltrami, the Cuban Minister of the Electrical Industry, it hopes to conclude similar agreements with the other member countries in the near future, and to establish a programme of multilateral cooperation.

● A new Comecon international organisation, Agroinform, has been established to coordinate scientific and technical information on agriculture and forestry. It is part of a

general policy of integrated information systems for the Comecon bloc, based on the International Scientific and Technical Information Centre of Moscow, to coordinate the



activities of member countries in collecting, selecting and processing information. So far Agroinform has dealt mainly with details of organisation and administration; this autumn, however, its International Council will meet in Prague to discuss the system's technical details. For the present exchange of agricultural information remains largely a matter of bilateral negotiations, such as the recent agreement between the Polish Academy of Sciences and the Czechoslovak Federal Ministry of Agriculture and Food covering research into the genetic and environmental factors affecting the quality of meat.

● During the past few years, Czechoslovak industry has made massive capital investments in plant and machinery purchased from abroad. By the end of 1976, some 15,000 million Czechoslovak crowns had been spent on equipment from 'capitalist' countries, a sum which theoretically is to be recouped by ever-increasing exports. But unfor-

tunately, some 58,000 million crowns worth of the imported equipment is still standing idle, due to delay in the construction of factories to house it. At one chemical plant, Chemiceluloza, 200 million crowns worth of imported equipment is reportedly waiting at the construction site and cannot be installed until late in 1978.

● A second Polish Antarctic research station will come into operation during the coming southern summer. Named the 'Antoni Boleslaw Dobrowolski' station, it was handed over to Poland by the Soviet Union in 1959 but has not been used until now because of a lack of funds. Situated at the Bunger Oasis, 300 km from the coast and 4,000 km from the existing Polish 'Henryk Arctowski' station on King George Island, it will conduct mainly geophysical and meteorological research sponsored by the Geophysics Institute of the Polish Academy of Sciences.

The opening of Dobrowolski station is part of a general increase of Polish activity in the Polar regions. After an intensive programme of geophysical, meteorological and oceanographic research at the Arctowski station last summer, a team of 19 scientists manned it for the present winter. Meteorological data from the station forms an important link in the weather-forecasting network based on the Soviet Molo-dezhnaya station.

● A detailed programme has been worked out for joint Comecon research into the use of solar energy. The programme is largely based on Soviet experience in this field; at present, about 100 small solar power stations are operating in the Soviet Union, and are expected to operate without interruption for more than 10 years. According to the Soviet newspaper *Trud*, these stations have one great disadvantage—the high cost of the electricity produced.

Vera Rich

IN BRIEF

JET suffering

The European Community's Joint European Torus (JET) fusion project, now costed at almost £100 million at January 1977 prices, is "overdue" and "suffering from serious lack of decision on vital matters", according to Bas Pease, the director of the Culham Laboratory near Oxford, where the JET design team is based. Writing in the laboratory's 1976 annual report, Dr Pease says that prospects of future European international cooperation depend on confidence that technically sound projects like JET can be carried out. The report also says that uncertainties over the site have made it difficult to plan certain work on JET.

Marine responsibilities

Those who believe that very few subjects are the specific responsibility of a single government department in Britain are vindicated by the contents of a publication prepared by the UK Government's Interdepartmental Committee on Marine Safety. Entitled *Marine Activities: Guide to the responsibilities of government departments and agencies*, the booklet shows, that marine pollution involving oil discharges, is the responsibility of the Department of the Environment when the discharges are from land or offshore installations within the 3-mile limit, but fall under the Department of Energy outside that limit. Occupational

health and safety are the responsibility of the Health and Safety Commission. The booklet is published by the Department of Trade.

IAEA's report

The growing uncertainty over the future of nuclear power in some industrialised countries is reflected in the International Atomic Energy Agency's annual report for 1976. It was partly responsible, the report claims, for orders for new nuclear plant falling from 53,000 MW in 1974 to 11,000 MW in 1976. The IAEA's total estimated regular budget for 1978 is \$48.8 million (£1=\$1.74). Expansion is planned for some areas of its work.

WITH the possibility of a world shortage of energy within the next twenty five years, many people are concerned lest this should jeopardise our food supplies. They are told that modern farming is 'energy intensive', producing fewer calories to eat than are expended in their production—in cultivations, manuring and harvesting—notwithstanding the stored solar energy obtained by photosynthesis.

It is sometimes therefore suggested, particularly by some who call themselves 'ecologists', that we should return to more primitive farming systems, or even to the practices of the pre-agricultural hunter and gatherer. The bushmen of the Kalahari desert in South Africa are often cited as examples of global frugality, living with the minimum disruption of the natural ecosystem, and taking out much more energy than they put into it. It is generally forgotten that this type of life is only possible with small and sparse populations; it is thought that the whole of Britain could only feed some five thousand people before the introduction of arable farming. The whole world might support about a million.

Without going so far back, it is perhaps worth looking at some of the farming methods used fifty or a hundred years ago. Thus if oil is getting scarce, it may be logical to use more horses. An analysis of the effect of such a change gives the surprising result that there would be an increase, not a decrease, in energy consumption.

In Britain today all the tractors, combine harvesters and other farm machinery use about 1% of our total oil consumption (under one million tons coal equivalent), even allowing for the fuel consumed in the factories making the machines. In 1939 we had

about a million working horses on our farms, but we would need at least double that number to harvest the vastly greater crops grown today. These horses would need to be fed every day of the year, not (like a

Frugal farming?



KENNETH MELLANBY

tractor) only when they were working. It used to be said that a horse needed three acres of land for its support, but much of this was probably rough grazing. Nevertheless they would need some grass, and possibly as much as two tons of oats, or its equivalent in other grain, per horse per year. This would mean that more than two million acres of the best farm land would be needed to support the horses, and so would be lost for the production of human food.

The energy equation is equally disastrous. The calorific value of the

horses' food would be at least four times that of the fuel powering the modern tractors, which do the work so much more quickly, and so enable the farmers to take advantage of Britain's changeable weather. In a bad year it is likely that, working with horses, much of the harvest would never be gathered in, and the land would seldom be cultivated in time for the next sowing.

Then if we went back to horse power, the human workforce would need to be doubled or trebled. Some people would welcome this, believing that it might decrease unemployment and reinvigorate village life. But are we prepared to pay all these farm workers the sort of wages they would expect from industry, when there would be a fall, rather than an increase, in food production? And where would they live? We have virtually abolished the 'tied cottage' reserved for farm workers; the houses are occupied by commuters, the retired and weekend visitors. On a purely arable farm the employees can travel to work, but where animals, including horses, are kept, the staff must live close at hand.

The main reason modern farming appears to use so much energy is not its machinery, but the inefficient food conversion of its intensively kept livestock, which waste over 90% of the food they eat. Our farm animals consume about forty times the energy used by our tractors. Horses, like other animals, are poor converters of energy. The surprising thing is that our highly mechanised farming techniques in Britain use only some 3% of our total energy budget. If yields are to be maintained, a return to old techniques and to the use of horses would greatly increase the input of energy down on the farm.

news and views

The return of whole-mantle convection

from Peter J. Smith

'THOUGH it has been clear for ten years that the motion of plates must involve flow in the mantle below, nothing is yet known with certainty about the form this motion takes.' This recent remark from McKenzie (*Geophys. J.*, **48**, 211; 1977) is indisputably true but conceals some interesting swings of opinion. When mantle convection was first proposed by Holmes (*Trans. Geol. Soc. Glasgow*, **18**, 599; 1931) and others it was clearly envisaged as a process taking place throughout the whole of the region between the crust and core. By the 1960s, however, when the hypotheses of continental drift and seafloor spreading were being combined and extended into the theory of plate tectonics, the idea of whole-mantle motion had already fallen into considerable, though not complete, disfavour. Some Earth scientists had produced what they felt were valid reasons for supposing the lower mantle to be immobile, and others had greatly increased our knowledge of the thin mobile layer immediately below the lithosphere. It was perhaps inevitable, therefore, that the asthenosphere should come to be regarded by many workers as the key to the lithospheric movements above.

But a year or so ago the idea of convection throughout the mantle (or, more correctly, throughout that part of the mantle below the lithosphere) began to regain favour; and this trend continues. Gough (*Earth planet. Sci. Lett.*, **34**, 360; 1977), for example, has come down in favour of whole-mantle convection on the basis of a study of the geoid. When departures of the geoid from the flattened Earth spheroid are plotted on a sphere, a single zero-elevation contour is found to divide the surface into two nearly equal zones with positive and negative elevations respectively. Moreover, these two zones are interleaved rather like the strips which cover a tennis

ball. The areas covered by the zone of positive elevation include the western Pacific, most of Australia, the Arctic, the North Atlantic-European region, western South America, the South Atlantic and the South Indian Ocean. The areas covered by the zone of negative elevation, on the other hand, include much of Asia and the Indian Ocean, the Antarctic, North America, the Brazilian shield, the eastern Pacific and the western Atlantic.

What does this antisymmetric pattern mean? To Gough it suggests mantle-wide convection, the scale of the two zones being too great to be attributable to the effects of near-surface structure. As Gough envisages it, upward currents in the mantle would exist beneath all areas of negative elevation (low geoid), there would be downward currents beneath areas of positive elevation, and horizontal flow would occur across the zero-elevation contour (one way near the surface and the other way at the base of the mantle). In other words, by this interpretation the geoid represents 'whole-mantle convection in a single three-dimensional cell.'

If this is right, it then raises the question of the extent to which mantle-wide convection influences the motion of the lithosphere. Gough sees some 'encouraging relationships' between his model and actual plate behaviour; for example, the model predicts rising currents beneath the East Pacific rise and downward flow beneath the western Pacific subduction zones. On the whole, however, he is inclined not to expect close correlations between global convection and plate motion, arguing that lower mantle behaviour will be partially decoupled from the lithosphere by the intervention of the asthenosphere. In other words, the existence of mantle-wide convection would not preclude shorter wavelength convection in the asthenosphere with perhaps greater influence on the overlying plates.

But this rather defensive posture is not shared by Davies (*Geophys. J.*, **49**, 459; 1977) who claims it to be 'unlikely that the lower mantle is *not* involved in motions related to plate tectonics.' Davies, too, draws attention to the possibility of small-scale flow in the upper mantle but he clearly regards such motions as perturbations on large-scale mantle-wide convection, thus imputing to them relatively less importance than does Gough. It would be misleading to make too much of this difference in emphasis, for both authors share the conviction that the limitation of convection to the upper mantle is unlikely. Davies is able to be somewhat more positive, however, because his conclusion is based on the study of whole-mantle convection models which, although simplified, can account qualitatively for the large horizontal scale of plates, the imperfect correlation between plate speed and the amount of attached descending slab, the motion of plates with no attached slab, and the opening of the Atlantic in particular.

What Davies's study of simple layered-fluid models shows is that thermal convection would be confined to the upper mantle only if the lower mantle were at least 10,000 times more viscous than the upper mantle. And even if the mantle were to play only a passive part in plate dynamics, the flow induced by the moving plates would fail to penetrate the lower mantle only if the viscosity of the lower mantle were at least 1,000 times greater than that of the upper mantle. Moreover, a shallow low-viscosity layer would have to be about 10,000 times less viscous than the mantle beneath for flow to be confined to it. In other words, it would appear that mantle-wide flow, whether induced thermally or by moving plates above, can only be prevented by extreme viscosity contrasts in the mantle.

Traditionally, of course, such contrasts have been widely accepted, or, as Davies puts it, 'speculations on and

models of mantle flow associated with plate tectonics have been dominated by the concepts of an elastic, high-viscosity lithosphere, a relatively low-viscosity asthenosphere, and a high-viscosity mesosphere.' Indeed, as far as the high-viscosity lithosphere and lower-viscosity asthenosphere are concerned, the tradition persists; the existence of these features is not seriously in doubt. What is at issue, however, is the viscosity of the lower mantle and its contrasts with, on the one hand, the viscosity of the asthenosphere and, on the other, the viscosity of the rest of the upper mantle below the asthenosphere.

To cut a long story short, several reasons have been adduced in the past for supposing the viscosity of the lower mantle to be many (for example, five or more) orders of magnitude greater than that of the asthenosphere, a view which appeared to have majority support until quite recently. But as Cathles's extensive studies have shown (*The Viscosity of the Earth's Mantle*, Princeton University Press, 1975), there are apparently equally good reasons to suppose that the contrast is much less. Cathles's own version is that the mantle has a fairly uniform viscosity of about 10^{22} poise throughout, apart from a thin channel (the asthenosphere) in the uppermost mantle which has a viscosity of about 0.4×10^{21} poise. It is difficult to say whether or not this view of mantle viscosity has become the new consensus; what can be said without fear of dispute is that the low-contrast mantle is now more popular than it was a few years ago and that it is now more fashionable to debunk arguments in favour of the high-viscosity lower mantle.

This clearly strengthens the case for mantle-wide convection, especially in the light of Davies's results. But what of the other traditional arguments against whole-mantle convection? In a very useful review section Davies looks at all the main ones and concludes in the light of recent information that none is of compelling validity. It has been suggested many times in the past, for example, that there may be chemical composition gradients in the mantle involving changes in mean atomic weight—obviously a situation which would be difficult to reconcile with the mixing brought about by whole-mantle convection. But as Mao (*J. geophys. Res.* **79**, 5447; 1974) and others have shown, the particular observations which led to the invocation of atomic weight changes can be explained at least as reasonably in other ways. Obviously this does not rule out the existence of chemical non-uniformity but it weakens the ability of such non-uniformity to stand

as an argument against mantle-wide convection.

The evidence for the presence of phase transitions in the mantle, on the other hand, is much stronger, which raises the questions of whether phase boundaries can persist in the face of convective mixing and of whether the persistence of such boundaries may prevent convective flow across them. The available information here is conflicting, for much depends on the precise conditions in the mantle. Davies concludes, however, that the case for saying that phase transitions will prevent whole-mantle convection is suspect at best, although such transitions could well modify the pattern of convective flow. Likewise, the case for excluding the lower mantle from involvement in plate dynamics on the grounds, among others, that earthquakes do not occur below about 700 km is also dubious, for there are ways of explaining this lack of seismicity without assuming that descending lithosphere fails to reach beyond this depth.

In summary, then, Davies has presented what is probably the most convincing case yet in favour of convection throughout the mantle below

the lithosphere, or at least the most convincing case for not rejecting whole-mantle convection quite so easily as it has often been rejected in the recent past. Nor is he alone. The support from Gough has already been mentioned, but there is more. Jones (*J. geophys. Res.*, **82**, 1703; 1977), for example, has suggested that long-term variations in the frequency of reversal of the geomagnetic field may be the result of fluctuating temperatures at the core-mantle boundary caused by convection in the lower mantle. And perhaps more importantly, Dziewonski *et al.* (*J. geophys. Res.*, in the press) are about to show that the large-scale seismic velocity anomalies detected in the mantle, including the lower mantle, by Niazi (*Bull. Seismol. Soc. Am.*, **63**, 2035; 1974) and others correlate with long-wavelength components of the gravity field—an observation which O'Connell (*Tectonophysics*, in the press) will relate to mantle motions. It would seem that the few people (for example, Run-corn) who have maintained their belief in mantle-wide convection for many years, if not decades, are to be vindicated, or at least are to see their views become fashionable again. □

Bed bugs and hepatitis

from Arie J. Zuckerman

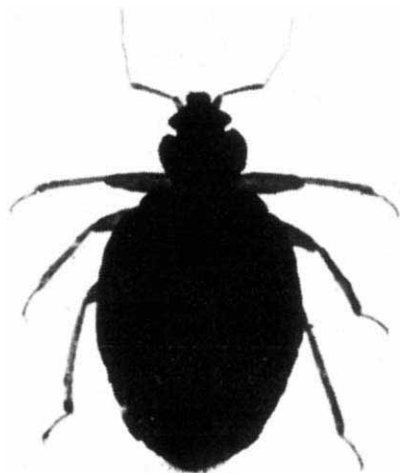
ALTHOUGH much has been written during the past few years on all aspects of human viral hepatitis, and hepatitis B in particular, it seems that this infection still has some epidemiological surprises to spring. The latest is a report by Willis *et al.* (*Lancet* **ii**, 217; 1977) that the bed bug could be a vector of hepatitis B.

With some 120–176 million carriers worldwide, the importance of hepatitis B cannot be overemphasised. In regions such as northern Europe, North America and Australia, where infection is relatively uncommon (a carrier rate of ~ 1 in 100), transmission is chiefly through inoculation of blood and blood products, although hepatitis B can also be transmitted sexually and in other body fluids such as saliva, menstrual discharge, breast milk and semen. In areas where infection is relatively common such as parts of tropical Africa, South East Asia and the Far East (with a carrier rate of 10–20% of the population), trans-

mission by direct inoculation of blood or blood products is not enough to account for the higher rate of infection. The modes of transmission of hepatitis B in the tropics are similar to those in other parts of the world, but additional factors may be of importance including traditional tattooing and scarification, ritual circumcision with unsterile instruments and repeated biting by blood sucking arthropod vectors, and in particular mosquitoes (or flying-pins; *Nature* **239**, 72; 1972) and bed bugs.

Review of the available evidence on the role of biting insects in the spread of hepatitis B shows that the results of preliminary investigations are conflicting. Hepatitis B surface antigen, which is one marker of the virus, has been detected in several species of mosquito, which have been trapped in the wild or fed on infected blood artificially, but no convincing evidence has been obtained either of replication of the virus or of its persistence after digestion of the blood meal. The very real possibility of mechanical transmission by mosquitoes nevertheless exists, for example as a result of interrupted and consecutive feedings particularly in areas with a high prevalence of hepatitis B (*Tech. Rep. Ser. Wld Hlth Org.*,

Arie J. Zuckerman is Professor and Director of the Department of Medical Microbiology and the WHO Collaborating Centre for Reference and Research on Viral Hepatitis at the London School of Hygiene and Tropical Medicine.



The bed bug, *Cimex lectularius* which lives in cracks and crevices in the house, especially in beds.

Photo: C. James Webb, London School of Hygiene and Tropical Medicine.

No. 602; 1977). Bed bugs live more intimately with man than do mosquitoes, take blood meals and could transfer blood and hepatitis B virus from one occupant of a bed to another. Brotman and her colleagues (*Lancet* **i**, 1305; 1973) collected bed bugs from dwellings occupied by prostitutes in the Ivory Coast and detected hepatitis B surface antigen in 1 (5.6%) of 18 pools of engorged bed bugs of the species *Cimex hemipterus*. In a laboratory study, Newkirk *et al.* (*Gastroenterology*, **69**, 982; 1975) artificially fed *Cimex lectularius* bed bugs on blood from a patient with acute hepatitis B. Hepatitis B surface antigen remained detectable in the bed bugs for over 4 weeks, and juvenile bed bugs contained antigen after moulting, at a time when the bug usually starts to search for a host and will refeed. In the course of another investigation, carried out in Senegal by Wills *et al.* (*op. cit.*) bed bugs were collected on four separate occasions from bedding in the huts of villagers. All the bed bugs were identified as *Cimex hemipterus*, the predominant species in West Africa and other tropical areas. Hepatitis B surface antigen was detected in unengorged nymph and adult bed bugs in each of the first three collections. Three out of 28 specimens were antigen-positive in the first collection and 3 of 17 specimens were positive in the second collection. In the third collection, 6 out of 9 bed bugs were positive when the bed occupant was known to be hepatitis B surface antigen-positive. The antigen was also detected in 3 out of 89 bed bugs of the fourth collection kept alive without a blood meal for 30 days. Of particular interest was the finding of the *e* antigen, which is a marker of infectivity of

hepatitis B virus (see *News and Views* **263**, 374; 1976), in 1 engorged and 1 unengorged bed bug. These newest reports are the highest field infection rates with markers of hepatitis B virus reported so far in any insect species. Clearly bed bugs feeding on the occupants of the same bed could amplify the risk of hepatitis B infection, and

this mechanism may provide an unromantic form of connubial spread of infection (Blumberg *Science* **197**, 17; 1977). There is undoubtedly a need to examine closely and objectively these and other blood-sucking insects, and the entire matter deserves further epidemiological and experimental investigation. □

Multispecific antibodies

David S. Secher

THE classic work of Landsteiner (*The Specificity of Serological Reactions*, Dover, New York, revised ed., 1962) in the first half of this century demonstrated that antisera have a unique specificity. In addition to recognising and discriminating between most foreign molecules, a man (or other vertebrate) can recognise newly synthesised organic chemicals which could not have been encountered during evolution. It has often been assumed that the exquisite specificity of antisera is a reflection of a similar degree of specificity in the antibody molecules that make up that antiserum. This, of course, would require the potential production of a number of antibodies equal to the number of specificities (perhaps 10^6 – 10^8). An alternative explanation of the high specificity of antisera which has been favoured by a number of immunologists was stated particularly clearly by Talmage in 1959 (*Science* **129**, 1643). He suggested that each constituent antibody species in an antiserum (a normal antiserum is almost invariably a complex mixture of many different antibody species) is capable of binding to several different antigens. The pattern of such cross-reactions will be different for different antibodies and each one will be effectively 'diluted out' in the whole antiserum. The only strong binding will be to the antigen that all the antibodies can recognise, namely the original, immunising antigen. In this way a highly specific antiserum may be made up from a population of much less specific antibodies. More recently Inman has developed this hypothesis in considerable mathematical detail (in *The Immune Systems* (eds Sercarz, Williamson & Fox) Academic, New York, 1974).

Until recently there was little experimental data to favour this hypothesis and the observation of more than one

antigen binding to an antibody (or to a myeloma protein) was assumed to arise from structural similarities between the two antigens (see for example Michaelides & Eisen *J. exp. Med.* **140**, 687; 1974). Over the past few years Frank Richards and his collaborators at Yale University School of Medicine have shown that the binding of two antigens (dinitrophenyl (DNP) and vitamin K_3) to the mouse myeloma protein MOPC 460 takes place on two spatially distinct sites in the immunoglobulin molecule. They have argued that this is therefore an example of multispecificity of the immunoglobulin and not due to a cross reaction between the antigens (Richards *et al.* *Science* **187**, 130; 1975). Richards *et al.* extended the observation of multispecificity to include antibodies present in immune rabbit sera and, by the elegant use of isoelectric focusing in polyacrylamide gels, showed that a second antigen could also be 'functional' in the triggering of lymphocytes to produce antibodies (see Richards *op. cit.* for review). Again the binding of other antigens to anti-DNP antibodies was studied.

How much of a contribution to the repertoire of antibody specificities could such multispecificity make? Richards *et al.* estimated that 100 haptens (antigens) might bind to a single antibody. We have used a more conservative 'degeneracy factor' of 10 in discussing antibody diversity (Secher, Adetugbo & Milstein, *Immunol. Rev.* in the press). But these numbers are still just guesses and data from other systems are clearly required to indicate how general the phenomenon is.

Cameron and Erlanger (this issue of *Nature* page 763) have now examined the binding specificities of antibodies to adenosine-5'-phosphate (AMP) (see also *Immunochemistry* **13**, 263; 1976). Three fractions of anti-AMP antibody were purified by preparative isoelectric focusing from the serum of rabbits immunised with AMP-gramicidin S. The ability of these fractions to bind

David Secher is a member of staff at the MRC Laboratory of Molecular Biology, Cambridge, and a Fellow of Gonville and Caius College.

antigens other than AMP was studied by the inhibition of enzyme immunoassays and by three other binding or cytotoxicity assays. In contrast to Richards *et al.* who tested more than 300 ligands to find only 2, Cameron and Erlanger appear to have found at least 2 cross-reacting antigens out of the 11 tested. Vitamin K₃ (yet again!) and caffeine were found to bind to two out of three of the antibody fractions.

Cameron and Erlanger draw an analogy between the multispecificity of antibodies and the binding to enzymes of ligands unrelated to the main substrate. Some enzymologists might disagree with interpretation of the enzyme data but in any case the analogy is not very appropriate. Enzymes are homogeneous and so multispecificity in substrate binding could not enhance their specificity in the way that Talmage, Inman and others have proposed for antisera.

There is one practical consequence of the multispecificity concept that has so far escaped comment. Monoclonal antibodies produced by the cell fusion technique (Kohler & Milstein *Nature* **256**, 495; 1975) might have unexpected cross reactivities. No such observation

has been reported but it is probably too early for anyone to have systematically looked for one.

A more fundamental question that has received surprisingly little attention is the relationship between antibody affinity and multispecificity. The 'best' antibodies bind their antigens with avidities of 10^{-9} – 10^{-7} l mol⁻¹ but so far multispecificity has only been observed in the $\geq 10^{-6}$ l mol⁻¹ range. It is clear from all the published data that as one examines antigens that bind more weakly to an antibody, there is a greater degree of cross reaction with other antigens. How much biological significance do these weaker interactions have? A normal immune response develops from low affinity IgM antibodies on the surface of cells in the primary response to high affinity IgG antibodies in the secondary response. Perhaps *in vivo* there is no single 'degeneracy factor' but a spectrum ranging from multispecific, low-affinity antibodies that enable an individual with relatively few cells to recognise a maximum of antigens, to monospecific, high-affinity antibodies. The best monoclonal antibodies may thus be highly specific after all! □

High-energy nucleus-nucleus collisions

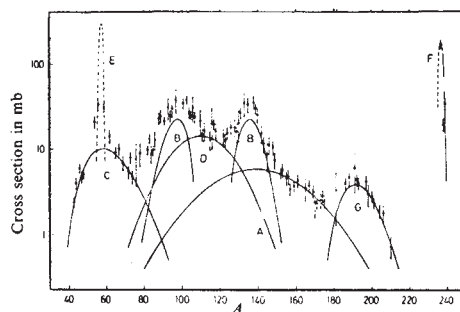
from P. E. Hodgson

VERY many complicated processes can take place when two heavy nuclei collide at high energies, and these can be studied in a global way by measuring the mass distribution of the reaction products. A sufficient proportion of these products is radioactive, so this can be done by a radiochemical analysis of the target after irradiation. Such analyses give only the total cross sections for the production of the various fragment nuclei, and thus complement the detailed studies of individual reactions to particular final nuclear states.

Among the cross sections that can be determined by this method are those for fusion, when the two nuclei fuse together to form a compound nucleus, and quasi-fission, in which several tens of nucleons are transferred from one nucleus to another by a deep inelastic collision. Previous studies of the interaction of ⁴⁰Ar with ²³⁸U and of ⁸⁴Kr with ²³⁸U show that in the former case 55% of the total reaction cross section may be ascribed to fusion and 9% to quasi-fission, while in the latter there is only 4% fusion and 38% quasi-fission. These results suggest that as the

mass of the projectile increases the probability of fusion falls rapidly. This is of great practical importance for those who are trying to make superheavy nuclei by the collisions of heavy nuclei since it shows that the heavier the interacting nuclei the less likely they are to stick together, implying that heavy-ion experiments designed to produce superheavy nuclei are considerably less feasible than has been previously supposed.

As it is very important to verify this conclusion and confirm that this trend is indeed a universal one, a further



Mass yield curve for the fragments from the interaction of 538 MeV ⁵⁶Fe with ²³⁸U, analysed into components corresponding to various reaction mechanisms as described in the text.

measurement of the reaction products from the interaction of ⁵⁶Fe with ²³⁸U has recently been made by Reus and collaborators from the Universities of Marburg and Manchester (*Phys. Rev. Lett.* **39**, 171; 1977). They find that 14% of the total reaction cross section is due to fusion, and 26% to quasi-fission, in line with the previous results.

The experiment was made by bombarding a target of ²³⁸U with 538 MeV ⁵⁶Fe ions from the Manchester Linear Accelerator. After irradiation, the target was analysed radiochemically and the cross sections for the production of 173 nuclides were determined. The results are shown in the figure as a function of atomic mass, and show a number of distinct peaks which can be attributed to particular processes. Thus component A is assigned to fusion reactions (followed subsequently by fission) because the form of the mass yield curve in this region is consistent with that of a broad Gaussian distribution peaking at a mass value of 137 ± 2 , which is the estimated most probable mass for fusion-fission products in this interaction.

The component B has a shape and location indicating that it is due to low energy asymmetric fission following the quasi-elastic transfer of a few nucleons from one nucleus to the other. Component D is obtained by subtracting A and B from the total mass-yield curve, and its maximum corresponds to the mass number to be expected from the symmetric fission of nuclei formed by the deep inelastic transfer of several tens of nucleons between target and projectile, and so it is assigned to fission following quasi-fission, or sequential fission. Component G corresponds to those nuclei that are formed by deep inelastic transfer from target to projectile and survive sequential fission, and component C is the light quasi-fission partner. Finally the two sharp peaks at either end of the distribution, components E and F, are due to quasi-elastic transfer. They are not very well determined because many of the nuclides in these mass regions have half lives that are unsuitable for radiochemical assay. As a check on the whole analysis, the total cross section calculated for this interaction agrees with the sum of all the individual cross sections.

Integration of the cross section corresponding to each component enables the relative importance of the various processes to be found. The type of interaction that is likely to lead to the formation of superheavy nuclei is fusion of the interacting nuclei to form a compound system. This is of course expected to be rather unstable, so it soon breaks up, probably by fission. The important thing is that the compound system lives long enough for its

properties to be studied, and this is not the case for any of the other processes identified in this analysis.

This experiment, together with the two previous ones already mentioned, gives fusion cross sections of 620, 190 and 55 mb for the interactions of Ar, Fe and Kr with uranium, showing that

the higher the mass of the projectile the lower the fusion cross section. Experiments designed to produce super-heavy nuclei will thus have to rely on reactions initiated by projectiles much lighter than the target, and this severely reduces the range of nuclei that might be produced. □

Meteorite research old and new

from Robert Hutchison

The Fortieth Annual Meeting of the Meteoritical Society was held at Cambridge, UK on 24–29 July, 1977. The successful meeting attracted a dedicated band of 'amateurs' together with astronomers, astrophysicists, chemists, geologists, mineralogists and physicists.

REPRESENTATIVE of the 'old' is the search for isotopic anomalies in meteorites and their interpretation in terms of the birth of the Solar System. This is contrasted, for me, with 'new' studies on the asteroids of today, with new experimentation on meteorites and with the discoveries of new meteorites.

Representative of classic meteorite research was the discussion over the interpretation of ^{26}Mg excesses in meteorites. Over the past few years, this ^{26}Mg excess (from decay of ^{26}Al , half life, 740,000 years) has been interpreted in two main ways. One school considers that the excess was introduced into the Solar System in pre-solar refractory grains in which ^{26}Al was already extinct (D. D. Clayton, Rice University, Houston); the other considers that ^{26}Al was present in a pre-solar nebula, condensation of which produced high temperature mineral aggregates in which ^{26}Al decayed to ^{26}Mg ; in these ^{26}Mg is proportional to the $^{27}\text{Al}/^{24}\text{Mg}$ ratio—the isochron relationship established by G. J. Wasserburg and co-workers at Caltech.

At the meeting Clayton argued that, because stars seem to form in cold gas-dust clouds, it must be assumed that all refractory elements were condensed at planet formation in the Solar System. Ca–Al–Ti-rich inclusions in the Allende meteorite, are, therefore, condensates from an expanding supernova. This is supported by the presence of volatile-rich rims around some inclusions; at least some of the rims predate incorporation into the meteorite. This argument was taken further by Schramm, Grossman and

others (University of Chicago), who now propose that the products of two supernovae were ejected into the matter from which the Solar System formed. From the earlier came the r-process nuclides (^{244}Pu and ^{129}I for example) with half lives long enough to allow survival over about 10^8 yr, until the second, smaller supernova produced, among others, ^{26}Al . Enrichment in ^{16}O was due to destruction of ^{17}O and ^{18}O in the second event. The two stage theory is based on lack of correlation between isotopic anomalies in the light elements (O, Ne and Mg) and on isotopic homogeneity in the heavy elements (Sr, U).

A probably important discovery was discussed by R. N. Clayton, who, with T. K. Mayeda (both from the University of Chicago), found that the hydrated matrix of the unusually metal-rich carbonaceous chondrite, Renazzo, has terrestrial oxygen isotope ratios: for the first time we have a potential source for our oceans and atmosphere. Of similar importance is the discovery of a strongly heated carbonaceous chondrite, 'Mulga West' from Western Australia. Few ordinary chondrites contain water, most having been heated to temperatures of about $1,000^\circ\text{C}$; in contrast, all previously known carbonaceous chondrites have low temperature mineralogy and so are often considered as originating in comets from the outer Solar System. The findings of R. A. Binns (University of Western Australia) and Australian and British coworkers now indicate that at least one carbonaceous chondrite parent-body was heated to the same extent as the (presumably) asteroidal parents of the ordinary chondrites. The question of the sources of meteorites was addressed by C. R. Chapman (Planetary Science Institute, Tucson). Considerable spectral and dynamic data have been accumulated on the asteroids, which can now be identified as potential sources of most meteorite types. However, problems still remain in relating the relative abundances of the different meteorite types to the relative abundances which could be produced from the asteroids.

Also in the 'new' category was a search for meteorites in Antarctica by W. A. Cassidy (University of Pitts-

burgh), E. Olsen (Field Museum, Chicago) and K. Yanai (Japanese Institute of Polar Research). With National Science Foundation support they spent two months near McMurdo Base. Their success in finding 11 meteorites has probably sparked off a new era in meteoritics, for it showed that the finding of hundreds of meteorites by the Japanese in the Yamato Mountains was not just a flash in the pan. And meteorites found in 'blue ice' areas should be sterile, a fact noted by the organic chemist C. Ponnamperna (University of Maryland), who did not give his paper as scheduled but was reportedly hunting meteorites in Greenland. Meteorites are normally named after places near where they are found; the lack of named geographical features in Antarctica therefore poses a serious problem in meteorite nomenclature. This was discussed by a Committee on Meteorite Nomenclature of the Meteoritical Society, whose conclusions will be communicated in due course.

Other papers of note included one by N. M. Evensen and colleagues (Lamont Observatory, New York) who confirmed earlier suspicions that rare earth elements (REE) in some chondrites are fractionated. This could be a blow to geochemists who normally use REE in chondrites as an 'unfractionated' standard for comparison with terrestrial rocks. A consortium study on the largely unshocked achondrite Serra de Magé provided a further enigma. M. Prinz and G. Harlow (American Museum of Natural History, New York) and their various collaborators showed that this stone cooled more slowly than any known basaltic meteorite; that its pyroxene minerals are similar to those in the huge, slowly cooled Stillwater intrusion of Montana. However, G. Lugmair and others (University of California, San Diego) used the Nd–Sm age determination technique to show that Serra de Magé minerals probably did not become closed systems until 4.52 Gyr ago. By that time several related meteorites had been cool, and sometimes fractured (by impact?), for over 100 Myr. This poses problems about the size and heat-source of the parent body.

The meeting did not concern itself with meteorites alone. Two sessions were on lunar studies and one covered 'Terrestrial Impact, Craters and Tektites', the first three papers of which dealt with a large Russian impact structure (49°N , 59°E). It was a pity that no Russian attended the meeting, but western collaborators described this Zhamanshin structure as an impact crater some 15 km in diameter, with associated natural glasses related to tektites.

Finally, several papers relied on new,

R. Hutchison is a Principal Scientific Officer in the British Museum (Natural History), where he is in charge of the Collection of Meteorites.

improved technology and experimental techniques. Data from the ion probe were presented and in one study of a fine-grained iron meteorite a scanning device on a transmission electron microscope was used to obtain high resolution spot analyses across the interface between α (low-Ni) and γ (high-Ni) metal. J. I. Goldstein and coworkers at Lehigh University, Pennsylvania intend to apply this technique to experimentally produced structures in an attempt to measure diffusion rates at low temperatures (below about 600 °C). □

More light on enzyme control

from C. J. Lamb and R. K. Dudley

It has been said in these columns that 'Plant molecular biology is not dead, but is merely awaiting the attention of competent and dedicated biochemists', (*News and Views* 254, 13; 1975). A recent illustration of the potential contribution that biochemical studies can make to the understanding of plant biology is given by the work of Klaus Hahlbrock and colleagues at the University of Freiburg on the photocontrol of phenylalanine ammonia-lyase (PAL) levels in parsley cell cultures. By looking at the production of PAL in cell-free systems they have been able to show that the increase in PAL production observed after illumination is due to an increase in translatable mRNA and not to activation of previously synthesised enzyme.

PAL catalyses the deamination of L-phenylalanine to trans-cinnamic acid, the first step in the biosynthesis of plant compounds based on the phenylpropane unit and including lignins, flavonoids and coumarins. Its activity increases greatly following illumination of various plant tissues and the enzyme has proved popular for studying the photocontrol of gene expression in higher plants. Unfortunately, many of these investigations have relied on the effects of the antimetabolites actinomycin D and cycloheximide, both of which have many different effects in higher plants. Furthermore, density-labelling studies of the phytochrome-mediated increase in PAL activity in mustard seedlings have produced conflicting results and there is considerable controversy as to whether in this case enhancement of PAL activity is caused by activation of previously synthesised

protein or by increased *de novo* synthesis. Hahlbrock's contribution has been to complement antimetabolite and *in vivo* labelling experiments with a careful study of the synthesis of PAL in cell-free systems. The results obtained to date suggest that this approach will give insight into a number of fundamental problems in plant biology.

Early work (reviewed by Hahlbrock *Physiol. Veg.* 14, 207; 1976) demonstrated that on irradiation with blue or ultraviolet light, previously dark-grown parsley cell suspension cultures accumulate a number of flavonoids concurrent with a large increase and subsequent decrease in the extractable activity of PAL. These responses are red/far-red reversible, but neither red nor far-red light has an effect without ultraviolet preirradiation, suggesting that the initial responses are not phytochrome-mediated. The ability of light to enhance PAL activity declines progressively, and after a few hours illumination further irradiation has no stimulatory effect. *In vivo* studies with actinomycin D and cycloheximide and labelling with ³⁵S-methionine and ¹⁵N-ammonia indicated that PAL was synthesised *de novo* following illumination. The light-mediated changes in the apparent rate of synthesis of the enzyme were calculated from the time-course of changes in extractable PAL activity under various programmes of illumination, and the results interpreted as reflecting short-term light-mediated increases in the concentration of specific mRNA available for translation.

This hypothesis has now been investigated directly by study of the biosynthesis of PAL in cell-free systems. *In vitro* protein synthesis with polyribosomes from parsley cell cultures was relatively low with a homologous system, but an effective heterologous system was established by supplementing the parsley ribosomes with an extract from wheat germ (Schröder & Hahlbrock *Biochim. biophys. Acta* 366, 454; 1974). The rate of synthesis of PAL was determined by incorporation of ³⁵S-methionine into protein precipitable by a rabbit antiserum prepared against the purified enzyme (Schröder, Betz & Hahlbrock *Eur. J. Biochem.* 67, 527; 1976). Analysis of the immunoprecipitate by disc gel electrophoresis in the presence of dodecylsulphate revealed small amounts of the complete enzyme subunit (molecular weight 83,000) and relatively large amounts of shorter peptides which were also immunologically characteristic of the enzyme; an extra check by tryptic fingerprinting would have been desirable.

The capacity for PAL synthesis was much greater with polyribosomes from

irradiated cells compared with those from dark-grown cells and the observed changes in the rate of PAL synthesis closely corresponded with those previously calculated from changes in the level of extractable PAL activity. The possibility that the increased capacity for PAL synthesis reflects changes in the amount or activity of ribosome-bound factors rather than changes in the level of translatable mRNA has been ruled out by extracting the RNA from parsley polyribosome preparations with chloroform/phenol and using this extract as a message in a rabbit reticulocyte lysate (Ragg, Schröder & Hahlbrock *Biochim. biophys. Acta* 474, 226, 1977). Synthesis of PAL could only be detected using RNA from light-treated cultures. This is the first report that a mammalian cell-free system will accept a plant message for translation, and is especially interesting since PAL is an enzyme of plant secondary metabolism and is not found in animals.

These studies demonstrate an increase in the level of translatable PAL mRNA in response to illumination, but is this a result of more message being produced, or less being destroyed, or to pre-existing message being made available for translation, or indeed to a combination of these possibilities? To answer this will require purification of the PAL mRNA, a daunting task as the message is likely to be only a tiny proportion of the total RNA extracted. However, enhancement of the level of PAL activity is accompanied by concurrent increases in the subsequent enzymes of the pathway and using Hahlbrock's approach it should be possible to analyse the mechanisms whereby coordinated gene expression in higher plants is achieved in response to environmental stimuli. Of immediate interest is the phytochrome-mediated stimulation of phenylpropanoid biosynthesis in intact plants. The regulatory mechanisms may well differ from those observed with cell cultures undergoing rapid cell division and nucleic acid biosynthesis. □



A hundred years ago

THE *Frigorifique*, fitted up for the transportation of meat on the Tellier system with methylic acid, has arrived at Havre, from Brazil, with its cargo in an excellent state of preservation. It is stated that a banquet of the meat will be served during the forthcoming session of the French Association at Havre.

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Chris Lamb is in the School of Botany at the University of Oxford and Keith Dudley is in the Department of Biochemistry at the University of Cambridge.

review article

Why the genome does not congeal

J. Maynard Smith

* School of Biological Sciences, University of Sussex, Falmer, Brighton, Sussex BN1 9QG

Since the genes in any given organism have evolved together to produce an adaptive phenotype, one might expect that rearrangement by chromosomal recombination would be maladaptive and thus wiped out by natural selection. This article reviews various theories that explain why, on the contrary, recombination is almost universal.

TEN years ago, Turner¹ asked "Why does the genome not congeal?" That is, why do organisms not evolve a single large chromosome, with no chiasmata, so that genes inherited from one parent, father or mother, are passed on without recombination to a child. This question is related, of course, to that of why organisms reproduce sexually rather than parthenogenetically². The argument against recombination, crudely, is this: if an individual has survived to breed, it is likely to carry a set of genes which combine well together, and recombination will therefore have the effect of breaking up sets of co-adapted genes.

In fact, there are good theoretical reasons for expecting natural selection to reduce recombination. Thus suppose that a population has reached genetic equilibrium under selection in a uniform environment; that is, gene frequencies at all loci have reached their stationary equilibrium values under selection. Then either the population will be genetically monomorphic, in which case recombination is selectively neutral, or (as almost all natural populations are) it will be genetically polymorphic at many loci. In the latter case, selection is likely to be for reduced recombination.

Fisher³ was the first to reach this conclusion. Serious mathematical work was started by Kimura⁴, and has recently been reviewed by Lewontin⁵. The crucial question is whether there is 'linkage disequilibrium' in the population: that is, whether the alleles at different loci occur independently of one another. (Technically, suppose there are two alleles at each of two loci, say *A*, *a* and *B*, *b*. Let p_{AB} be the frequency in the population of the gamete *AB*, and similarly for p_{Ab} , p_{aB} and p_{ab} . Then the 'coefficient of linkage disequilibrium' $D = p_{AB}p_{ab} - p_{Ab}p_{aB}$; linkage equilibrium requires that $D = 0$.) The relevance of D is simply that genetic recombination brings a population closer to linkage equilibrium; if $D = 0$, recombination does nothing, and there is no selection for higher or lower recombination.

Selection, however, can maintain linkage disequilibrium in a population provided that (1) the loci concerned are reasonably close (that is, do not recombine too freely), and (2) there are 'epistatic' fitness interactions between them. 'Epistasis' means that the effect of substituting an allele at one locus depends on what allele is present at the other. If, for example, *AB* and *ab* were of high fitness and *Ab* and *aB* of low fitness, this would be an extreme example of epistasis. Alternatively, one could say that *AB* and *ab* were 'co-adapted' sets of genes, and that *Ab* and *aB* were not. Given such fitness interactions, it is easy to see that the frequency of *AB* and *ab* gametes will tend to be high and of *Ab* and *aB* gametes low (that is, the population will be in linkage disequilibrium), and also that there will be selection for lower recombination, because recombination will

tend to produce *Ab* and *aB* gametes. In other words, either there is no selection on recombination, or selection will reduce it. This is what led Turner to ask his question.

Before seeking an answer, I first describe an experiment to measure the strength of selection. In *Drosophila*, it is possible (by using inversions and relying on the fact that there is no recombination in the male) to breed flies whose chromosomes are identical to ones present in adult wild-caught flies, and to compare their viability and fertility with flies whose chromosomes have undergone a single round of recombination since their extraction from adult wild-caught flies. Charlesworth and Charlesworth⁶ performed such an experiment, and found a small (and statistically non-significant) reduction in viability, and a larger and statistically significant reduction in fertility. This measure of the 'recombinational load' confirms the theoretical prediction that selection favours a reduction in recombination. Yet recombination is almost universal. Three kinds of explanation have been suggested.

(1) Chiasmata are needed to ensure proper disjunction in meiosis, and genetic recombination is an unselected byproduct. It is hard to believe this. Male Diptera manage an effective meiosis without chiasmata. Even if this were difficult to evolve, localisation of chiasmata at the ends of chromosomes, where they would have little genetic effect, would surely be possible.

(2) Recombination accelerates evolution to meet changing conditions; species which lose or reduce recombination go extinct in competition with those which retain it. The snag with this long-term 'group selection' theory is that whenever people have looked for genetic variance in recombination rate⁷⁻¹³ or chiasma frequency¹⁴ within a species they have found it. So if there is short-term selection for reduced recombination it will be effective, whatever the long-term consequences for the species. Nevertheless, this type of explanation is assumed in many discussions of the evolution of recombination rates.

(3) There is some short-term selection for higher recombination, because real populations are not in genetic equilibrium in a uniform environment. It is with theories of this third kind that this article is concerned. They are of three kinds, and allow for the fact that the environment is unpredictable, that relatives compete with one another, and that linkage disequilibrium will arise by chance in finite populations.

An unpredictable environment

It is widely assumed that if the environment is unpredictable, so that offspring meet selective forces different from those acting on their parents, then it will pay a parent to produce recombinant gametes. I have shown² that this is true only if the

environment is unpredictable in a special and rather implausible way. Consider an environment with a number of variable features, A_m or a_m , B_m or b_m and so on (these can be thought of as hot/cold, wet/dry and so on), and a population with alternative alleles A or a , B or b adapting individuals to different features. Then it can be shown that, even with random dispersal of offspring and changing frequencies of the alternative states between generations, any selection on recombination will favour a reduction. In particular, if alleles at two different loci, say A_1 and A_2 , adapt an individual to state A_m , and alleles a_1 and a_2 to a_m , then there will be selection for reduced recombination.

Only in the very extreme case of unpredictability, in which the correlations between environmental features—say A_m with B_m and a_m with b_m —change sign between generations will there be selection for increased recombination; that is, only if hot places tend to be dry in one generation and to be wet in the next.

This conclusion may be counter-intuitive. If so, I think it is from anthropomorphism. We argue 'I must not put all my eggs in one basket, because I might lose them all.' But in competition between two types, say H and L for high and low recombination, what matters is not that each individual should have at least one surviving offspring, but whether individuals of type H or L have on average the larger number of surviving offspring. In these circumstances, it pays to put all your eggs in one basket if it is a better-than-average basket.

In this analysis², I considered frequency changes for one generation only, for a rather artificial selection regime. A more realistic genetic model was considered by Charlesworth¹⁵, with essentially similar results. He considered a population in an environment which fluctuated either cyclically or stochastically, and found that the allele for higher recombination increased in frequency only in those simulations in which D_m (the value of D which would have been reached at equilibrium if environmental conditions were held constant) changed sign from time to time; this in effect requires that correlations between selectively relevant features should change sign, as in my model. He also found that the periodicity (or autocorrelation) of the environmental change was important.

These models of an unpredictable environment do show that there can be short-term selection for increased recombination. My own judgement of them, however, is that they show that the widespread belief that environmental unpredictability is sufficient to account for recombination is wrong, because the type of unpredictability required is of a kind which may seldom occur.

Suppose, however, that there is spatial variation in the environment. A very simple model is illustrated in Fig. 1. A haploid sexual population inhabits an area divided into 3 regions, in which genotypes AB , Ab and ab respectively are able to survive, other genotypes being lethal. Selection acts before dispersal of the haploid individuals, which may then migrate as shown in the figure, and which then fuse in pairs and immediately produce a new haploid generation. Recombination

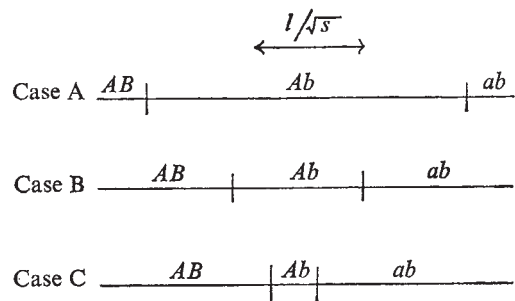


Fig. 2 Relation between the typical migration distance, $l/\sqrt{s}$, and the distance between environmental discontinuities. AB , Ab , and ab are the fittest genotypes in the respective regions.

is irrelevant in the terminal regions. In the central region it is advantageous in the double heterozygotes AB/ab , since it enables them to produce viable Ab offspring.

Slatkin¹⁶ analysed a more realistic model (Fig. 2). He considered a linear habitat with two boundaries, at one of which selection switches from favouring allele A to a , and at the other from B to b . If the boundaries are far apart (case A), two independent clines arise; there is no linkage disequilibrium and no selection on recombination. If they are coincident or close together (case C) there will be linkage disequilibrium either side of the boundaries, and selection for reduced recombination.

If the boundaries are an intermediate distance apart (of the same order as the 'characteristic distance' $l/\sqrt{s}$, where l is the typical distance an individual moves, and s the selective advantage per locus) there will be selection for higher recombination between the boundaries (case B). Hence for a spatially varying environment there can be selection for increased recombination. Unfortunately it is equally easy to think of situations (case C) in which selection favours reduced recombination.

Competition between sibs

Williams^{17,18} has pointed out that if sibs compete with one another it will pay a parent to produce a genetically variable family. Computer simulation¹⁹ shows that this can be a powerful force favouring sexual as against asexual reproduction or high as against low recombination. The basic model is shown in Figure 3. To fix ideas, imagine a population of self-incompatible hermaphrodite plants. The environment is divided into 'patches', each large enough to support one adult plant. Each patch has a number of features—5 in the simulations—each of which can be in one of two states, A_m or a_m , B_m or b_m ... E_m or e_m . In each generation the states of each patch are randomly assigned, the 32 possible types being equally common.

Individuals are diploid, with 2 alleles at each of 5 linked loci, A or a , B or b and so on. Genotypes AA and Aa are equally adapted to A_m , and aa to a_m ; the other loci are similarly related to other features. Thus an individual can be adapted to 0, 1... 5 features of the environment.

There are L patches, and hence L surviving adults. To produce a new generation, a new set of L patches is generated randomly. Each patch receives N seeds from each of R female parents. To produce N seeds, 2 parents are chosen randomly and offspring produced according to the usual laws of genetics. The 5 loci are linearly arranged, and the rate of recombination between them is determined by alleles at a sixth modifier locus.

When RN seeds have been produced in a patch, one survivor is chosen randomly from those best adapted to it. A successful parent, therefore, is one who produces many winners. Since there can be only one winner in each patch, it pays to produce as variable a family as possible. Since recombination increases within-family variability, alleles for high recombination increase in frequency.

Some results of simulating the model are given in Table 1.

Fig. 1 Migration in a spatially heterogeneous environment, giving selection for higher recombination in the central region.

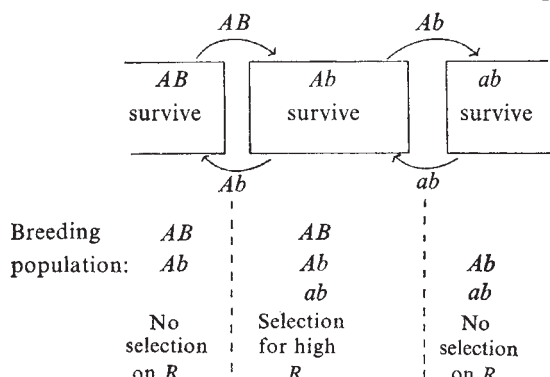


Table 1 Selective advantage of recombination

Population size, L	Parents per patch, R	Offspring per parent, N	Total selection intensity, NR	Recombination fractions		Number of generations	Frequency of allele C^+		Estimated selective advantage of C^+/C^+
				C^-	C^+		Initial	Final	
200	4	8	32	0.0	0.5	15	0.265	0.462	+0.165
200	5	10	50	0.05	0.5	10	0.262	0.387	+0.181
400	5	10	50	0.05	0.5	6	0.330	0.426	+0.182
400	5	10	50	0.05	0.25	6	0.301	0.388	+0.189
400	5	10	50	0.05	0.25	6	0.305	0.355	+0.115
Locus of C unlinked to fitness loci									
400	5	10	50	0.05	0.5	6	0.316	0.375	+0.127
400	5	10	50	0.05	0.5	6	0.272	0.325	+0.147

There is a substantial advantage for recombination. In contrast to the models discussed in the next section, the advantage does not disappear if the modifier locus is unlinked to the fitness alleles.

Thus by modifying the 'unpredictable environment' model to allow for competition between sibs, one obtains substantial short-term selection for higher recombination. There are, however, two reasons why sib competition cannot by itself explain why the genome does not congeal. First, the dispersal pattern of the species must be such that sibs compete with one another, yet meet environmental conditions different from those in which their parents survived. Second (as in the models discussed in the last section), if alleles at two loci adapt an individual to the same environmental conditions, selection will reduce recombination between them.

Hitch-hiking and the evolution of finite populations

'Hitch-hiking' is easiest to understand for genes which modify the rate of mutation. Suppose that a mutator gene causes a unique favourable mutation which increases in frequency and becomes fixed. If there is no recombination the mutator gene will also be carried to fixation. This theoretical possibility was discussed by Leigh²⁰, and Cox & Gibson²¹ showed that a gene for a high mutation rate was repeatedly carried to fixation in a non-recombining population of *E. coli*.

Strobeck *et al.*²² suggest that a similar process may select for alleles for higher recombination. Consider a particular case. A population has a balanced polymorphism for a pair of alleles A and a (for example, because Aa is fitter than AA or aa). A unique favourable mutation B occurs, say in an AB gamete. B will increase in frequency for a time, but if there is no recombination this increase will be interfered with by selection at the A locus. In the absence of aB gametes, B cannot go to fixation.

Suppose now that there is a pair of alleles C^+ and C^- at a third linked locus, such that recombination between A and B occurs only in C^+/C^+ genotypes. Sooner or later an aBC^+

gamete will arise by recombination, and will increase in frequency. The allele C^+ hitchhikes a lift from the aB gamete it has produced, but only if it can hang on; that is, only if C is closely linked to A and B .

A simulation is shown in Figure 4. In fact the allele C^+ receives two lifts. The first can be down (case A) or up (case B), according to whether the original B mutation occurred in repulsion or coupling with C^+ . The second, when an aBC^+ gamete is produced, is always upwards. The average effect is always to increase the allele for higher recombination.

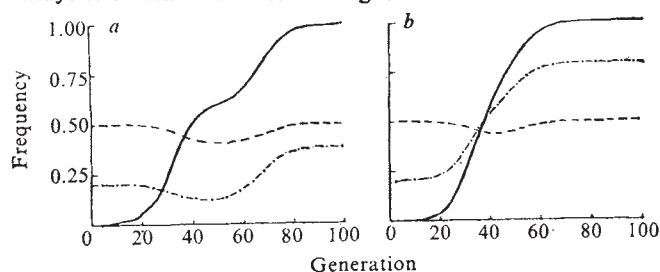


Fig. 4 The hitch-hiking effect on a gene for recombination: a, gene frequencies when B is initially in coupling with C^- ; b, gene frequencies when B is initially in coupling with C^+ . ---: frequency of A ; —: frequency of B ; — — —: frequency of C^+ .

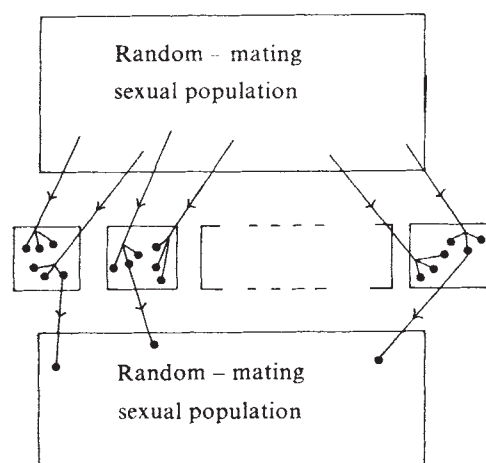
The process operates whenever evolutionary changes occur in a population with selectively maintained polymorphisms; to that extent it is universal. But how important is it? Strobeck *et al.*²² simulated a range of values of selection and recombination rates. The net effect, if any, is always to increase recombination. The main limitation is that the net increase in C^+ is vanishingly small unless, in the absence of C^+ , the three loci are closely linked. Hence the process explains why the genome does not congeal completely, but may not account for the high levels of recombination actually observed.

Hitch-hiking is a phenomenon of finite populations. In an infinite population AB and aB gametes would both arise by mutation. Felsenstein²³ pointed out that models showing a short-term or long-term advantage for sex are usually finite-population models. He suggested that this is because in a finite population linkage disequilibrium will arise by chance (Hill & Robertson²⁴). This led Felsenstein & Yukoyama²⁵ to simulate finite populations initially polymorphic for alleles affecting recombination, in which a succession either of favourable or of unfavourable mutations occurred. They observed selection for higher recombination in these populations. Two favourable genes interfere with one another just as a favourable and heterotic gene interfere. A modifier which can cause recombination between two favourable or two unfavourable alleles may get a lift from the favourable recombinants it has produced.

The remaining problem

Three types of model in which selection for increased recombination occurs have been considered. Models incorporating an unpredictable environment have two drawbacks; they require an extreme and implausible type of unpredictability, and they imply selection for reduced recombination whenever

Fig. 3 Dispersal pattern for the sib-competition model, with 3 offspring per parent, and 2 parents contributing to each patch.



several loci are concerned with the same environmental feature. Models incorporating sib competition overcome the first but not the second of these drawbacks; they introduce an additional requirement for a pattern of dispersal which, while not implausible, is certainly not universal.

Finite-population, hitch-hiking models have the required universality. They have the drawback that there is substantial selection only if the modifier locus is tightly linked to the loci affecting fitness. In effect, such models explain why the genotype does not congeal, but not why recombination is as free as it is. They will be most effective in populations which are continuously adapting to qualitatively new circumstances. Fisher³, who, characteristically, was the first to hint at a mechanism of this kind, wrote "It is apparent that for this process to have been an effective check upon the constant tendency to increase the intensity of linkage, the stream of favourable mutants must be an abundant one."

- ¹ Turner, J. R. *Evolution* 21, 645 (1967).
- ² Maynard Smith, J. *J. theor. Biol.* 30, 319 (1971).
- ³ Fisher, R. A. *The Genetical Theory of Natural Selection* (Oxford University Press, Oxford, 1930).
- ⁴ Kimura, M. *Evolution* 10, 278 (1956).
- ⁵ Lewontin, R. C. *The Genetic Basis of Evolutionary Change* (Columbia University Press, New York, 1974).
- ⁶ Charlesworth, B. & Charlesworth, D. *Genet. Res.* 25, 267 (1976).
- ⁷ Abdullah, N. F. & Charlesworth, B. *Genetics* 76, 447 (1974).
- ⁸ Allard, R. W. *Genetics* 48, 1389 (1963).
- ⁹ Catcheside, D. G. *Austr. J. Biol. Sci.* 28, 213 (1975).
- ¹⁰ Chinnici, J. P. *Genetics* 69, 71 (1971).
- ¹¹ Dewess, A. A. *Genetics* 64, 516 (1970).
- ¹² Dettelson, J. A. & Roberts, E. *J. expl. Zool.* 32, 333 (1921).
- ¹³ Kidwell, M. G. *Genetics* 70, 419 (1972).
- ¹⁴ Shaw, D. D. *Chromosoma* 37, 297 (1972).
- ¹⁵ Charlesworth, B. *Genetics* 83, 181 (1976).
- ¹⁶ Starkin, M. *Genetics* 81, 787 (1975).
- ¹⁷ Williams, G. C. & Mitton, J. B. *J. theor. Biol.* 39, 545 (1973).
- ¹⁸ Williams, G. C. *Sex and Evolution* (Princeton University Press, Princeton, 1975).
- ¹⁹ Maynard Smith, J. *J. theor. Biol.* (1976).
- ²⁰ Leigh, E. G. *Amer. Natur.* 104, 301 (1970).
- ²¹ Cox, E. C. & Gibson, T. C. *Genetics* 77, 169 (1974).
- ²² Strobeck, C., Maynard Smith, J. & Charlesworth, B. *Genetics* 82, 547 (1976).
- ²³ Felsenstein, J. *Genetics* 78, 737 (1974).
- ²⁴ Hill, W. G. & Robertson, A. *Genet. Res.* 8, 269 (1966).
- ²⁵ Felsenstein, J. & Yokoyama, S. *Genetics* 83, 845 (1976).

articles

Antarctic circumpolar current: space and time fluctuations in the Drake Passage

D. James Baker, Jr

Department of Oceanography, University of Washington, Seattle, Washington 98195

Worth D. Nowlin, Jr

Department of Oceanography, Texas A & M University, College Station, Texas 77843

R. Dale Pillsbury & Harry L. Bryden

School of Oceanography, Oregon State University, Corvallis, Oregon 97331

New oceanic data on the structure and variability of the Antarctic Circumpolar Current (ACC) in the Drake Passage and its interaction with the atmosphere have revealed a more complex spatial structure than has been seen before. They also show a significant coherence between surface wind and deep current at short periods. These new results will be essential in understanding the role of processes in the polar regions in global climate dynamics.

OCEANIC data have been obtained from two sets of closely spaced density sections and a year-long moored current meter array carried out during two international (U.S., Argentina, Chile, U.S.S.R.) multi-ship operations in the Drake Passage (between South America and the Antarctic Peninsula) in January–March 1975 and January–March 1976. These expeditions (FDRAKE: First Dynamical Response and Kinematic Experiment) are part of the International Southern Ocean Studies (ISOS), a component of the International Decade of Ocean Exploration of the Intergovernmental Oceanographic Commission of UNESCO. The atmospheric data are from an analysis of surface pressure for 1975 provided by the Australian Bureau of Meteorology in Melbourne. The data will be discussed in greater detail in several papers to be published elsewhere^{1–4} but we present here a summary of the major results on the structure of the flow field and its response to atmospheric and bottom topography interactions.

The primary purpose of FDRAKE 1975–76 was to begin to

establish a 'climatology' of the ACC system in the Drake Passage by the use of monitoring sections of current meter arrays and closely spaced hydrographic measurements. The mooring arrays were deployed and recovered by the current meter group from Oregon State University; and the hydrographic and chemistry work was carried out by groups from Texas A & M University and Oregon State University with technical support from the Scripps Institution of Oceanography. The hydrographic work in 1975 (refs 5–7) and 1976 (refs 8–10) included measurements of temperature, salinity, oxygen, phosphate, silicate, nitrate, and nitrite. When combined, these data provide a sharper picture of water masses and their characteristics in the regions adjacent to the Drake Passage, and they facilitate interpretation of data from the moored arrays. Data from the cruises were combined to give a nearly synoptic picture of the polar front zone from the Drake Passage to the Falkland Plateau and information on a second frontal zone, the Weddell–Scotia confluence. The work on the polar front zone is discussed in detail elsewhere^{2,3}.

A banded spatial structure is unambiguously revealed by the closely spaced hydrographic measurements⁴. In 1975, the stations occupied by the RV Melville were spaced 50 km apart, and in 1976, the station spacing was 45 km, in contrast to the previous work which had spacings generally greater than 100 km. This increased resolution has revealed vertically coherent bands of isopycnal slope steeper than the average downward slope of isopycnals towards the north, so that the density field has a step-like appearance.

The general characteristics of all the closely spaced sections are similar. These density steps produced cores of high geostrophic

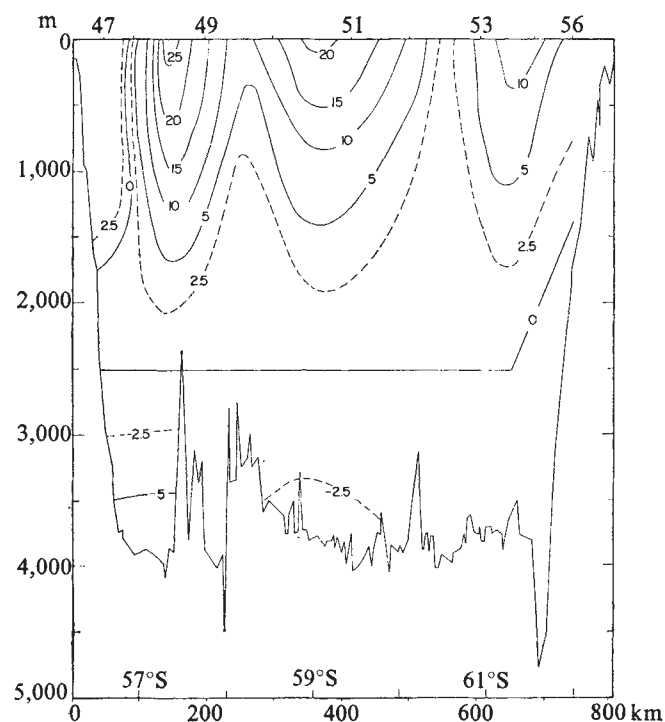


Fig. 1 Geostrophic velocity perpendicular to a line (running 332°T) across the Drake Passage (positive eastward). The flow is referenced to 2,500 dbar; south of 61°S, the greatest common sample depth was used as reference for station pairs which did not reach 2,500 dbar.

velocity shear. In 1975, the central Passage showed bands of eastward geostrophic speeds ranging from 25–30 cm s^{-1} separated by regions of lower speed (0–5 cm s^{-1}) or slight westward flow (Fig. 1). This core-like structure was observed again in March 1976 when a line of hydrographic stations was made from the RV Thompson approximately 25 km to the east of the current meter array. Three bands of large horizontal density gradient, or cores of relatively strong flow, were observed in this section. The detailed structure of these cores will be discussed elsewhere⁴.

Data from cruises of the Ob in 1958 (ref. 11), the Washington in 1969 (ref. 12) and the Hudson in 1970 (ref. 13) also provide some evidence for the bands of relatively high horizontal density gradients embedded in the current in Drake Passage. However, the station spacing on these cruises was too large to permit unambiguous description of the detailed current structure. Calahan¹⁴ has observed a similar structure, but with larger scales, in the ACC region south of Australia.

The characteristic scales of the high speed cores is the order of magnitude (20 km) to be expected if the fluid responds (to

atmospheric input, bottom topography interaction and/or instabilities) on the scale of the Rossby radius of deformation (defined as $[(g/\rho)(d\rho/dz)]^{1/2}H/(\Omega \sin\theta)$ where g is gravity, ρ density, H a characteristic depth (here chosen to be 1,000 m), Ω the Earth's rotation rate, and θ the latitude) averaged over the first 1,000 m. This is the first observation of fluid structure on this scale in this region, although mesoscale structure has been observed and suggested theoretically in other oceans¹⁵. J. S. Allen and M. McCartney (unpublished data) have shown that stratified, time-dependent flow through Drake Passage topography can lead to a core-like structure; we hope that these new data will stimulate further theoretical work on ocean response to these interactions in the southern ocean.

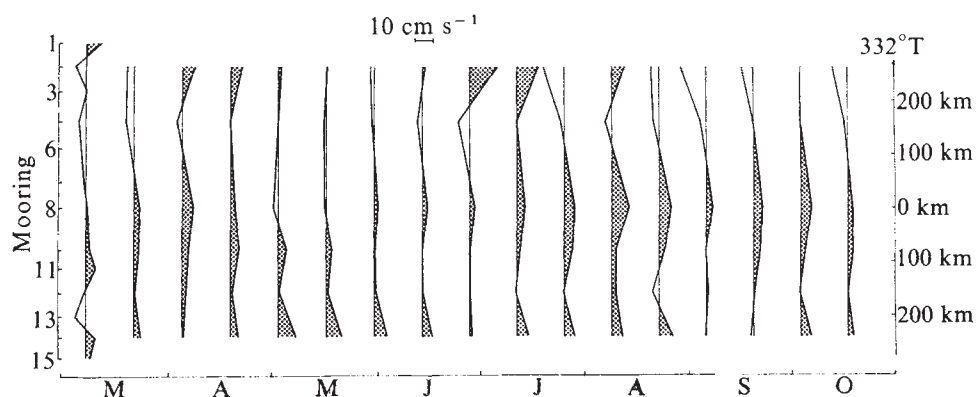
In February 1975, an array of 42 current meters on 15 moorings was deployed in the Drake Passage: 23 were recovered after three weeks, and 19 were recovered during February 1976, having measured currents over periods from 220 to 350 d.

These long-period records give us a unique data set for this region to define temporal and spatial statistics (previous measurement periods were a maximum of 13 d). To estimate time scales, an autocorrelation function for velocities in the through-passage direction (062°T) is calculated¹ for the long-term current record nearest 2,700 m. The period of the first zero-crossing of the autocorrelation function, which averaged 14 d, is an estimate of the time-scale of dominant energy containing motions. The period of independence of the records is estimated at 7 d. The 7-d averages of flow in the through-passage direction vary from –2.9 (westward) to +7.6 (eastward) cm s^{-1} over the measurement period of 1 yr. Only two of these mean flows are significantly non-zero at a 95% confidence level. Figure 2 shows the variation in the 14-d averaged through-channel flow over the year. A three regime structure is marginally present, reminiscent of the three-core hydrographic data. Note the strong changes on the northern side of the Passage. The low frequency variability is strongest on the northern side of the Passage, while the high frequency variability does not seem to change significantly over the width of the Passage.

The detailed statistics of this long-term variability of the deep flow will be discussed elsewhere¹. It is, however, interesting that the observed large variability in the flow at 2,750 m now seems to be one of the main reasons for the large range of transports estimated by various authors^{12,13} since in making these estimates short-term averages of deep currents were used to reference the baroclinic transports.

To estimate space scales, we note that the Rossby radius relevant to deep flow near the mooring number 10 (59°8'S, 63°4'W) is about 20 km. Thus, it is plausible that correlations of horizontal velocity at distances greater than about 40 km should be small¹⁵. This expectation is borne out by the statistical analysis of the current measurements¹ which show no significant correlations over the record lengths at the mooring spacing of about 75 km. The effect of this observed spatial variability on the total transport is discussed elsewhere^{1,4}. Significant coherences between moorings have been found for some events shorter than the record length, but

Fig. 2 14-d Averages of flow at 2,500 m from the current meter data, March–October 1975. The coordinate system is oriented in the cross- (332°T) and through- (062°T) passage directions. The shaded regions represent positive through-passage flow. During March a larger number of short-term moorings were deployed.



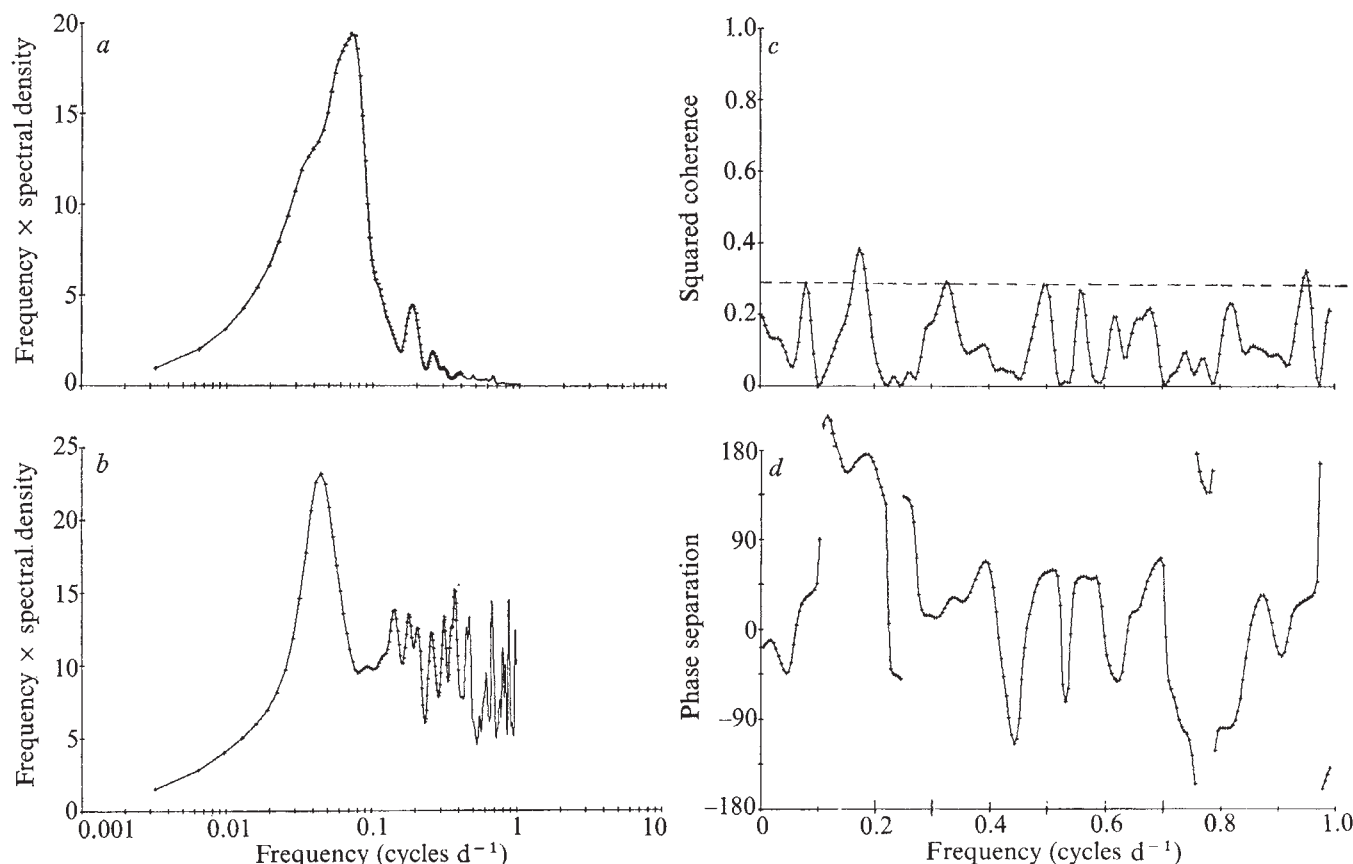


Fig. 3 *a* Spectrum of currents (at mooring 10: 1,519 m, 59°8'S, 63°4'W); *b* spectrum of geostrophic wind (components in the 062 °T, through-passage direction) in the Drake Passage (units are $\text{cm}^2 \text{s}^{-2} \text{cycles d}^{-1}$ per unit bandwidth (currents), $\text{m}^2 \text{s}^{-2} \text{cycles d}^{-1}$ per unit bandwidth (wind)); *c*, squared coherence of currents and wind; and *d*, phase of currents and wind. Time series are 308 d long, beginning 27 February 1975. See text for discussion of coherence near the 5.5-d scale. 95% confidence limits are indicated.

these are still preliminary studies. The current meter array in place during 1976 and that designed for 1977 have separations of smaller scales in order to examine velocity correlations more closely.

An analysis of the surface meteorological data has also been carried out for the Drake Passage; 12-h values of pressure and pressure differences have been extracted from the 12 months of 1975 surface pressure analysis. Ten values were used over an area $1,000 \times 1,000 \text{ km}$ centred on the moored array. Both the pressure spectrum and the pressure difference spectrum, interpreted as geostrophic wind, show a number of peaks in the range of periods from 2 to 14 d, with the bulk of the energy falling at long periods (greater than 14 d). The current meter spectra show peaks near 4 d and 6 d with the bulk of the energy falling between 10 and 100 d (see Fig. 3). Coherence calculations between the through-passage current at 1,019 m and 1,519 m on mooring 10 and the through-passage wind show a significant (at the 95% significance level) peak in the coherence squared at a period of 5.7 d with a stable phase over the peak of about 172° . This coherence is good evidence that atmospheric effects are transmitted deep into the ocean at these high latitudes. It is, of course, possible that this is not a local effect. Since the atmospheric spectra show similar features to the west of the Drake Passage, we may be seeing here an oceanic response which is representative of a larger-scale air-sea interaction in the South Pacific Ocean. The currents are nearly out-of-phase with the local wind, suggesting that a larger-scale interaction may be at work.

The observed coherence between deep ocean currents and wind agrees with calculations by Philander¹⁶ which show that at frequencies less than inertial, and for forcing at large horizontal scales, there can be a response even in the deep water. Philander notes that the large eastward travelling cyclones that dominate the

spectrum of surface winds in mid- and high-latitudes should force a response down to the ocean floor in such a weakly stratified region, and that measurements between Iceland and Scotland¹⁷ have shown a correlation between surface atmospheric pressure and deep ocean currents at depths from 300 to 1,000 m. By his calculation, a similar response should be seen in the Drake Passage (the stratification is weak enough and the storm systems are large enough). Therefore, he suggests that the observed variability of deep currents in the Drake Passage is correlated with atmospheric storms; the data tend to bear out this suggestion.

Another question of some interest for Southern Hemisphere air-sea interaction is the existence of semi-annual wave components in the current meter data, which can be compared with the well known semi-annual atmospheric oscillations¹⁸. Surface ocean data, from sea level and ship drift observations, have shown such a variation¹⁹. A wave fit to the data from all the FDRAKE moorings shows significant amplitude (defined as greater than twice the standard deviation) at this period at all depths. The magnitudes of the semi-annual component range from 5.7 cm s^{-1} at a depth of 1,019 m to 0.9 to 1.3 cm s^{-1} at a depth of 2,750 m for the through-passage component. The cross-passage component also shows a significant amplitude, with a maximum at the shallower depth. This data set is the first which is long enough to show evidence of such long-term response in the deep water. The possibility of such deep forcing by wind adds a new facet to the complexity of air-sea interaction in high latitudes.

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- ¹ Bryden, H. L. & Pillsbury, R. D. *J. phys. Oceanog.* (in the press).
² Gordon, A. L., Georgi, D. T. & Taylor, H. W. *J. phys. Oceanog.* (in the press).
³ Joyce, T. & Patterson, S. L. *Nature* **265**, 131 (1977).
⁴ Nowlin, W. D. Jr, Whitworth III, T. & Pillsbury, R. D. *J. phys. Oceanog.* (in the press).
⁵ Gordon, A. L. *Antarctic J. U.S.* **10**, 142–143 (1975).

- ⁶ Nowlin, W. D. Jr, Patterson, S. L., Pillsbury, R. D. & Anderson, G. C. *Antarctic J. U.S.* **10**, 144–146 (1975).
⁷ Wear, R. B. Jr, & Park, P. K. *Antarctic J. U.S.* **10**, 141–142 (1975).
⁸ Nowlin, W. D. Jr, Pillsbury, R. D., Gordon, L. I., Anderson, G. C. & Baker, D. J. Jr *Antarctic J. U.S.* **11**, 154–156 (1976).
⁹ Joyce, T. M. *Antarctic J. U.S.* **11**, 157–158 (1976).
¹⁰ Patterson, S. L. & Sievers, H. A. *Antarctic J. U.S.* **11**, 158–159 (1976).
¹¹ Eskin, L. I. *Inform. Biull. sov. Antarkt. Eksped.* 29–32 (1959); English trans: *Sov. Antarct. Exped. Inf. Bull.* **2**, 52–55 (Elsevier, Amsterdam, 1964).
¹² Reid, J. L. & Nowlin, W. D. Jr *Deep-Sea Res.* **18**, 51–64 (1971).
¹³ Foster, L. A. thesis, Univ. Dalhousie (1972).
¹⁴ Callahan, J. E. *J. geophys. Res.* **76**, 5859–5864 (1971).
¹⁵ Gould, W. J., Schmitz, W. J. Jr & Wunsch, C. *Deep-Sea Res.* **21**, 911–931 (1974).
¹⁶ Philander, G. *Ocean Model. Newslett.* (1976).
¹⁷ Meincke, J. *Int. Council Expl. Sea. C.M.* 1975, C:29 (Hydrography Committee).
¹⁸ Schwerdtfeger, W. & Prohaska, P. *Meteor. Rund.* **9**, 33–43 (1956).
¹⁹ Van Loon, H. *Tellus* **23**, 511–516 (1971); *Deep-Sea Res.* **19**, 525–527 (1972).

New Miocene locality in Turkey with evidence on the origin of *Ramapithecus* and *Sivapithecus*

Peter Andrews

British Museum (Natural History), Cromwell Road, London SW7, UK

H. Tobien

Johannes-Gutenberg-Universität, Saarstrasse 21, 6500 Mainz

Collections in early Middle Miocene deposits at Pasalar in Turkey have yielded a very rich fauna. Included in this are two hominoid species referred here to Sivapithecus darwini (Abel) 1902 and Ramapithecus wickeri (Leakey) 1962. These are both more primitive morphologically and earlier in time than other species of these genera, and they provide evidence that Sivapithecus and Ramapithecus are closely related and that their early diversification may have occurred not in Africa but in Eurasia.

THE mammal-bearing locality at Pasalar, Turkey, is situated 68 km south-west of Bursa, 12 km south-west of Kemalpaşa, and about 6.5 km south of the main Bursa–Susurluk road, NW-Anatolia (Fig. 1)¹. The site was discovered in 1969 during a lignite exploration² in the Neogene basins of Anatolia, and is situated near the Southern edge of the Neogene Gönen Basin (west of Bursa)³. The lignite survey

was inaugurated by the Turkish government, and reconnaissance trips and field work were done in 1965–1969 by a group of geologists from the Bundesanstalt für Geologie und Rohstoffe (Hannover, FRG) in collaboration with the Maden Tetkik ve Arama Enstitüsü (MTA, Ankara)². During the field work about 80 fossil vertebrate localities of late Neogene and early Pleistocene age were discovered. Some of these localities were subsequently investigated by a German research team under the leadership of the late Professor Sickenberg in collaboration with Turkish colleagues of the MTA and with the financial support of the Deutsche Forschungsgemeinschaft⁴.

Stratigraphy

The fossiliferous deposits at Pasalar are exposed in a small road cutting. They are resting directly on Palaeozoic granitic rocks which are clearly exposed on the west side of the road. The fossiliferous sediments consists of 2.2 m of silts and sandstones, often quite coarse, with a considerable thickness of apparently unfossiliferous silts and fine sandstones further up the section to the north and east.

The section exposed on the eastern wall of the roadcut, about 750 m south of the village of Pasalar, is as follows:

0.8 m grey-green silt, unbedded, with many small calcareous concretions, some rare irregularly distributed quartz grains, and many chips of bone of larger mammals and rare isolated teeth of micromammals.

1.0 m whitish to light grey sand, fine grained, unbedded, with frequent lenses of coarser sands and gravels (mainly quartz pebbles up to several centimeters in diameter), unbedded and badly sorted, non calcified. Abundant fossils.

0.4 m grey-green silt, with occasional small red spots, many cm-sized white calcareous nodules, unbedded, unfossiliferous.

Unconformity

Palaeozoic granitic rocks and undifferentiated metamorphosed rocks.

The relatively coarse sediments that make up the fossiliferous series are overlain by a finer grained more strongly bedded succession. Discontinuous beds and lenses of coarse sands, which decrease in frequency up the section, occur throughout. The depositional environment was fluvial, with rapid sedimentation in high energy conditions in the main fossiliferous bed followed by quieter conditions, but with occasional high energy episodes, later on. This accords well with the location of the sediments, situated as they are on the southern border of the Neogene Gönen Basin at the base of the sedimentary sequence. They probably represent initial molasse-like fill of a tectonic basin near the lower slopes of a mountain range⁴.

Fig. 1 Map of northwestern Anatolia showing the positions of Pasalar and Candir.

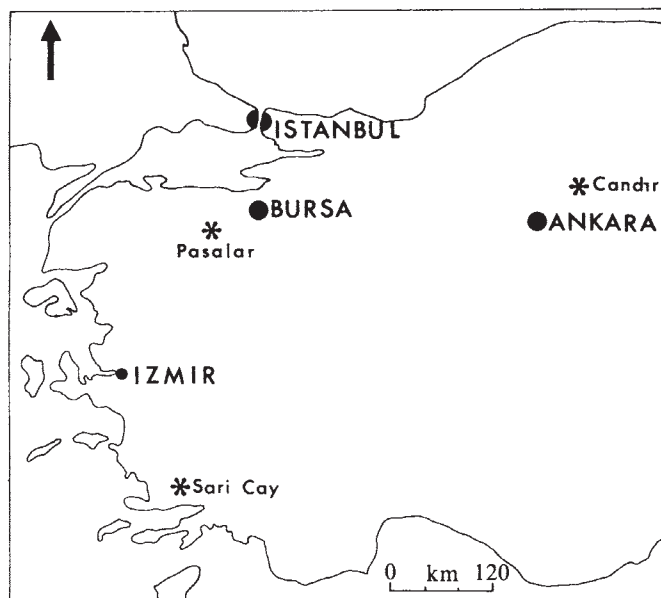


Table 1 Pasalar faunal list

<i>Sivapithecus darwini</i> (this work)
<i>Ramapithecus wickeri</i> (this work)
Soricidae sp. (small form)
Talpidae sp. 1
<i>Galerix</i> cf. <i>moedingensis</i>
Erinaceinae sp.
Chiroptera (middle sized form)
<i>Prolagus</i> cf. <i>oeningensis</i>
<i>Alloptox</i> n.sp. 1
<i>Cricetodon</i> (' <i>Palaeocricetus</i> ') sp.
<i>Megacricetodon</i> sp.
<i>Democricetodon</i> sp.
Sciuropteridae sp. indet.
cf. <i>Spermophilinus bredai</i>
<i>Pseudodryomys</i> sp.
cf. <i>Rhizomys</i> sp.
Ctenodactylidae sp. indet.
Dipodidae sp. indet.
<i>Amphicyon</i> cf. <i>major</i>
<i>Hemicyon sansaniensis</i>
<i>Plithocyon</i> sp.
<i>Ursavus</i> cf. <i>primaevus</i>
<i>Ursavus</i> aff. <i>intermedius</i>
<i>Plesiogulo</i> n. sp.
<i>Proputorius</i> sp.
<i>Trochictis</i> sp.
Lutrinae sp. 1
<i>Protictitherium intermedium</i>
<i>Protictitherium</i> aff. <i>gaillardi</i>
<i>Protictitherium</i> sp. 1
<i>Percrocuta</i> (<i>Percrocuta</i>)
miocenica
<i>Percrocuta</i> sp. 1
<i>Pseudailurus</i> cr. <i>quadridentatus</i>
<i>Pseudailurus</i> (<i>Schizailurus</i>)
<i>larteti</i>
<i>Orycteropus</i> sp.
<i>Deinotherium giganteum</i>
<i>Gomphotherium angustidens</i>
<i>Anchitherium</i> sp.
<i>Tapirus</i> sp.
<i>Bellajevina tekkayai</i>
<i>Listriodon splendens</i>
<i>Schizochocerus</i> sp.
<i>Conohyus</i> sp.
Cervidae indet.
<i>Protorix</i> cf. <i>caroliniae</i>
<i>Prostrepsiceros</i> cf. <i>rotundicornis</i>
Bovidae indet.

Fauna

The bulk of the fossil remains come from the sand and gravel layer. Within that bed the fossils are concentrated in the unconsolidated gravels and consist largely of isolated teeth of mammals ranging in size from insectivores to elephants. There are also many fragmentary postcranial elements, and these occur, together with the isolated teeth, in very great local concentrations.

The faunal list (Table 1) for the main fossiliferous bed is provisional except for the carnivores⁵, proboscidiens⁶ and rhinoceroses⁷. The description of the other groups is under preparation, and a preliminary account of the primates is given here. They are referred to two species of *Ramapithecus*⁸⁻¹⁰ and *Sivapithecus*¹¹⁻¹³ on the basis of morphological similarities with previously described species.

In the lithostratigraphic subdivision of the late Neogene in Western Anatolia, the Pasalar sequence represents an equivalent of the Middle Miocene Turgut member. More specifically its mammalian fauna can be correlated with the fauna from Prebreza (Yugoslavia)⁸, and it is somewhat earlier than the faunas of Bjelometscheskaya (Northern Caucasasia) and Sansan (France)¹. It is also one of the earliest of the Anatolian Miocene *Anchitherium* faunas¹. In the traditional European timescale Pasalar corresponds with the early Vindobonian⁸, slightly older than Sansan. In the biozonation of the Mediterranean Neogene based on mammals

it belongs to the NM zone 5 (ref. 14). In the sequence of European continental mammal ages/stages, proposed by the Symposium München 1975, the NM zone 5 is the uppermost member of the Orleanian age/stage¹⁵. In terms of the marine stratigraphy of the Paratethys and Tethys the Pasalar niveau would probably correspond to the Tharkanian (Eastern Paratethys), Lower Badenian (Central Paratethys) and Upper Langhian (Tethys) respectively. The radiometric age of the Langhian timespan is between 15.03 and 16.27 Myr (ref. 16).

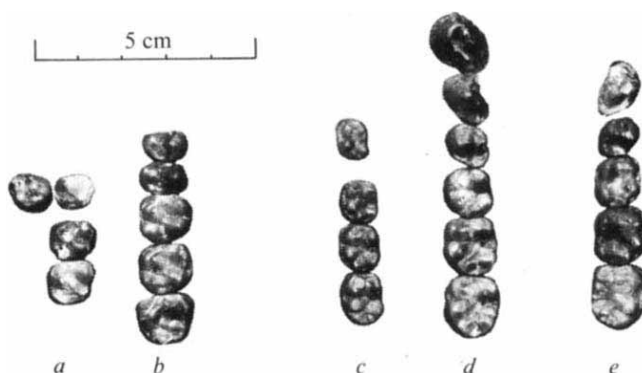
Fossil primates

The collection of primates from Pasalar consists of 100 isolated teeth: 14 of these are broken, allowing measurement of enamel thickness, but the rest are intact and well preserved. They group readily into two size categories, large and small, and on this basis a minimum number of individuals of 20 has been calculated, taking account also of the degree of wear of the teeth and whether they are from the left or right side, 12 for the large category based on 69 specimens, and 8 for the small category based on 31 specimens. Most of these are from the main fossiliferous bed, but three specimens are known from the overlying grey-green silt.

There are 11 incisors and canines in the collection. Five of these are upper central incisors, the large specimens having an expanded lingual pillar, so that they are intermediate in morphology between *Proconsul nyanzae* and *Sivapithecus indicus*, while the small incisors are similar in morphology to *Proconsul* incisors. In size the large specimens are equivalent to the I¹ of *Proconsul major* while the small ones are equivalent to very small *P. nyanzae*. (*Proconsul* here is considered to be a valid genus¹⁰.) The five canines are all from the left side and are very similar to each other both in size and morphology (Fig. 2d). They are the same size as *Sivapithecus indicus* canines, and it is assumed that they belong to the large size category.

There are 22 premolars in the Pasalar collection. The P³ is similar in size to P⁴ (Fig. 2b) and is not buccally elongated. The P⁴ is represented by eight specimens in the large category, all from the left side, and one specimen in the small size category. They are similar in morphology to *Sivapithecus* and *Ramapithecus* except that they are slightly broader relative to length. The P₃ samples are much more distinctive. The crowns are robust and low crowned and they have prominent mesiolingual cingula which end consistently in small-to-large beak-like processes (Fig. 2d, e). These are not usually seen in *Sivapithecus* species, although the size and degree of robustness of the crown is similar to

Fig. 2 Composite tooth rows of Pasalar primates. a, Left and right dp⁴ and left M¹-M² of *Ramapithecus wickeri*. b, Left P³-M³ of *Sivapithecus darwini*. c, Left dp₄ and M₁-M₃ of *Ramapithecus wickeri*. d, Left C-M₃ of *Sivapithecus darwini*. e, Right P₃-M₃ of *Sivapithecus darwini*.



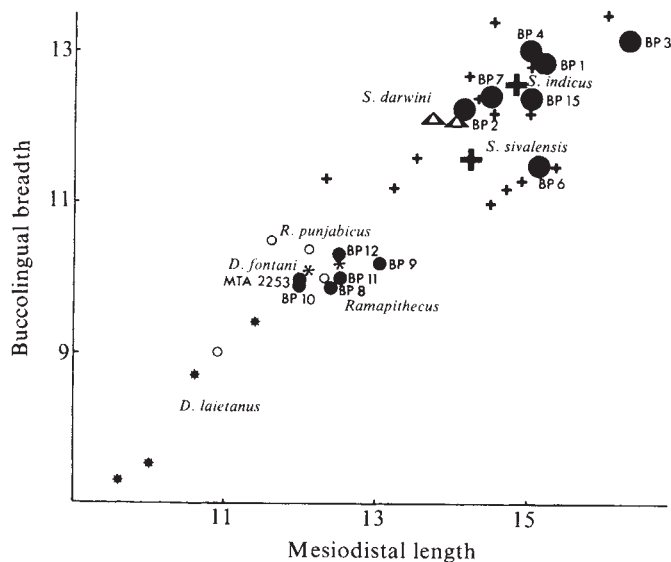


Fig. 3 Bivariate plot of M_3 . Black circles indicate the Pasalar specimens, the smaller ones representing the small specimens and the large ones the large specimens. BP, Bursa-Pasalar; MTA, Maden Tetkik ve Arama Enstitüsü, Ankara. Triangles indicate the type specimens of *Sivapithecus darwini*. The + signs indicate the Siwalik sample of *Sivapithecus* with the means for *S. indicus* and *S. sivalensis* taken from ref. 13 shown enlarged. The very small open circles represent *Ramapithecus punjabicus*. The stars represent small samples of *Dryopithecus laietanus* and *D. fontani*.

that of *Sivapithecus indicus*. The P_4 crowns are much more similar to those of *Proconsul* species, particularly their constricted trigonid regions and the presence of buccal cingula.

There are 18 upper and 32 lower molars from Pasalar. The size ranges in all the teeth are approximately equivalent to the ranges of *Sivapithecus indicus* for the large size category, and *Ramapithecus* species for the small category (Fig. 3). The upper molars are extremely similar in morphology to each other and to the molars of *Sivapithecus indicus*, and are quite unlike the molars of *Proconsul* species (Fig. 2a, b). This is mainly due to the presence on both of thick crown enamel which results in low rounded cusps, constricted trigonid basins, and blurred occlusal ridges. Enamel thickness on the molars varies from 1.7–2.5 mm in unworn teeth of the large size category to 1.1–1.5 mm on slightly worn teeth of the small size category. The lower molars also have low rounded cusps and constricted talonid basins due to greater enamel thickness, and in this they resemble *Sivapithecus* and *Ramapithecus*, but in general proportions the lower molars are more like those of *Proconsul*. This is partly due to the buccal cingulum, which is prominent (Fig. 2c, e) and imparts a squared outline to the crown of M_1 and M_2 , but also the trigonid is constricted and the M_3 has an elongated talonid narrowing distally. This *Proconsul*-like morphology of the lower molars contrasts with the *Sivapithecus*-like morphology of the upper molars, but there is little reason to doubt that the uppers and lowers at Pasalar are associated. Finally there are four deciduous teeth in the collection from Pasalar (Fig. 2a, c) all belonging to the small-size category.

Phylogenetic implications of fossil primates

The Pasalar primate sample of hominoid primates has been divided into two size classes (Fig. 3). They are too distinct to be the result of sexual dimorphism within a single species, but apart from that they are very similar to each other morphologically. There are, however, some morphological

differences between the two groups, particularly in the I^1 and P_3 , and we conclude, therefore, that they represent two different but closely related taxa. Both taxa are broadly intermediate in morphology between early Miocene *Proconsul* species and later Miocene *Sivapithecus* and *Ramapithecus* species, but they must be classified with the latter rather than the former because they share apomorphous (derived) characters with the later species of *Sivapithecus* and *Ramapithecus*, for example the thick enamel on the molars. The characters they share with the early Miocene species, such as molar cingula and narrow trigonids, are either primitive characters retained by both groups or synapomorphies present in all the dryopithecines.

The large species is the same size as *Sivapithecus indicus* from the Siwaliks¹³. But, it differs from *S. indicus* in many respects, particularly in the morphology of the central incisor, third premolar and lower molars, as well as being rather earlier in time. It also differs from *S. sivalensis* in many of the same ways, and most resembles *Sivapithecus darwini* (Abel)^{11,12}. This is a poorly represented fossil species known from deposits in the Vienna basin that are about the same age as the Pasalar deposits. The morphology and size of the primate specimens from the two localities are almost identical, and the Pasalar species is therefore referred to *Sivapithecus darwini* until further discoveries, especially of mandibular or maxillary material, make a more detailed assessment possible.

The teeth representing the small species from Pasalar are very similar to those of the mandible recently described from Candir⁸, Turkey. This has been referred to *Ramapithecus wickeri*⁹ on the basis of mandibular as well as dental morphology. The Candir deposits are similar in age to those of Fort Ternan, which is the type locality of *R. wickeri*, but the Pasalar deposits seem to be a little older, and related to this the Pasalar *Ramapithecus* is slightly more primitive than *R. wickeri* from Fort Ternan and Candir. On present evidence the difference is not considered great enough to justify a new species name for the Pasalar form, so it will be retained in *R. wickeri*.

Stratigraphically the Pasalar deposits are intermediate in age between the early Miocene deposits of East Africa and the later Miocene dryopithecine localities of Europe and Asia¹³. This raises the possibility that the primates from Pasalar, which have also been shown to be intermediate morphologically, may provide a phylogenetic link between early Miocene *Proconsul* of Africa and later Miocene *Sivapithecus* and *Ramapithecus* of Eurasia. In this event, the great degree of similarity exhibited by *S. darwini* and *R. wickeri* from Pasalar, which suggests a closer relationship between *Ramapithecus* and *Sivapithecus* than hitherto recognised^{10,13}, indicates that there may be represented at Pasalar a very early stage in the origin and radiation of these two genera. This radiation was almost entirely confined to Europe and Asia, with the exception of *R. wickeri* from Fort Ternan (and some doubtfully assigned specimens of *Sivapithecus*), and although the origin of this radiation will probably ultimately be traced to Africa, on present evidence it seems that its initial diversification took place outside of Africa and probably in Central Asia.

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- 1 Sickenburg, O. *Geol. Jber.* **B15**, 21 (1975).
- 2 Alpan, S. & Lüttig, G. *Newsl. Stratigr.* **1**, 11–18 (1971).
- 3 Lüttig, G. & Steffens, P. *Paleogeographic Atlas of Turkey* **1**, 1: 500,000, sheet Middle Miocene, Hannover (1976).
- 4 Tobien, H. *Eclogae geol. Helv.* **61**, 564 (1968).
- 5 Schmidt-Kittler, N. *Palaeontographica A* **155**, 117, 124 (1976).
- 6 Gaziry, A. W. *Geol. Jber.* **B22**, 13, 81 (1976).
- 7 Heissig, K. *Geol. Jber.* **B19**, 89 (1976).
- 8 Tekkaya, I. *Bull. Miner. Res. Explor. Inst., Ankara* **83**, 148 (1974).
- 9 Andrews, P. & Tekkaya, I. *Int. Cong. prehist. protohist. Sci.* **9** (in the press).
- 10 Andrews, P. *Int. Cong. primat. Soc.* **5** (in the press).
- 11 Abel, O. S. *Ber. Akad. Wiss. Wien, math.-nat. Kl.* **111**, 1171 (1902).
- 12 Lewis, G. E. *Am. J. Sci.* **34**, 145 (1937).
- 13 Simons, E. L. & Pilbeam, D. R. *Folia primat.* **3**, 81 (1965).
- 14 Mein, P. *Rep. Activity R.C.M.N.S. Work Groups, Bratislava*, 78–81 (1975).
- 15 Fahlbusch, V. *Newsl. Stratigr.* **5**, 164 (1976).
- 16 Vass, D. *Rep. Activity R.C.M.N.S. Work Groups, Bratislava*, 107 (1975).

A genetic model for sympatric speciation through habitat diversification and seasonal isolation

Catherine A. Tauber & Maurice J. Tauber

Department of Entomology, Cornell University, Ithaca, New York 14853

Based on studies with natural populations, we offer a genetic model for sympatric speciation that could have broad application to polyphagous animals with habitat differences. The model comprises three steps, each of which includes simple genetic changes and plausible selective forces.

ALLOPATRIC speciation as the almost universal, single mode of speciation in bisexual animals has been the subject of lively dispute¹⁻⁶. As an alternative, Maynard Smith⁷ proposed a theoretical model for sympatric speciation through disruptive selection in a two-niche situation. The details of this model were subsequently extended, especially for plants⁸.

Until now, the most convincing evidence supporting the application of Maynard Smith's model to bisexual animals comes from studies with monophagous insects that form divergent host races⁹⁻¹². In fact, Bush^{5,13} suggested that sympatric speciation through disruptive selection is restricted to host specific, phytophagous or parasitic animals. Far more controversial are cases of proposed sympatric speciation through, for example, diversification in habitat association or seasonal distribution^{1,3,14-16}. Unlike the cases involving host races⁹, there are no step-by-step genetic models for this type of speciation mainly because there is a lack of information on the inheritance of ecophysiological response patterns controlling habitat preference and seasonality in animals.

Work with two green lacewings, *Chrysopa carnea* Stephens and *Chrysopa downesi* Banks (Insecta: Neuroptera), has shown that single allele differences at two loci result in gross alterations in photoperiodic responses controlling seasonal timing of reproduction¹⁷. Also, allelic changes at three loci can explain the sympatric speciation of *C. downesi* from a *C. carnea*-like ancestor¹⁸. Based on these and other analyses of genetic differences between *C. carnea* and *C. downesi*, we present a genetic model for sympatric speciation through habitat diversification and seasonal isolation. This model involves three steps—each of which includes simple genetic changes and plausible forces of disruptive selection. We propose that our specific model has broad application to animals that differ from sympatric relatives in both habitat association and in reproductive season. We also show that Maynard Smith's general model⁷ for sympatric speciation is applicable to bisexual, polyphagous, as well as monophagous, animals.

Reproductive barriers

C. carnea and *C. downesi* readily hybridise in the laboratory and produce fully viable and fertile F₁ and F₂, as well as backcross offspring; in these conditions, there are no behavioural or post-mating reproductive barriers between the two species. But, no hybrids are found in nature and the two species are kept clearly separate through the action of two types of premating reproductive barriers—differences in their habitats and differences in their seasonal periods of reproduction¹⁹.

Reproductively active *C. carnea* adults, which are light green, occur in grasslands and meadows, in cultivated fields, occasionally on deciduous trees, and rarely on conifers. In these habitats, the adult coloration provides camouflage, presumably against vertebrate predators. At the end of summer,

as the adults enter reproductive diapause, they move to the senescent foliage or leaf litter of deciduous trees where they overwinter^{20,21}. This movement is accompanied by a colour change (from light green to reddish brown) that maintains the camouflage in the overwintering site. In contrast, *C. downesi* adults do not change colour and the species is restricted to coniferous trees, where the very dark green colour of the adults provides camouflage. *C. carnea*'s occasional occurrence on coniferous trees indicates a slight overlap in habitat with *C. downesi*; but, in general, the two species are spatially separated.

Adults of both species undergo diapause, during which reproductive behaviour, ovarian development, and sperm transfer are suppressed. *C. carnea* enters diapause at the end of August and it remains in diapause until mid-January or February²². Postdiapause mating takes place before the vernal equinox, and the first postdiapause generation adults emerge about June, after which two more summer generations are produced. Therefore, mating by *C. carnea* occurs during late winter and also during summer²³. In contrast, *C. downesi* has only one generation per year, and the adults, on emergence in June, enter a diapause that persists through summer, autumn and winter¹⁹. This species ends its diapause around the time of the vernal equinox²⁴; mating takes place in early spring. Therefore, *C. downesi* is reproductively inactive during *C. carnea*'s entire reproductive period.

The seasonal cycles of *C. carnea* and *C. downesi* are primarily controlled by photoperiod²⁵. Their asynchrony largely results from *C. downesi*'s short day-long day requirements for diapause prevention, and for diapause termination. These short day-long day requirements evolved from photoperiodic response patterns that *C. carnea* and *C. downesi* share, that is, responsiveness to a change in daylength and responsiveness to the actual duration of daylight (or darkness) per 24-h period^{17,25,26}.

The species-specific responses are under genetic control, and a pair of alleles at each of two loci regulate the expression of the *C. carnea* versus the *C. downesi* response pattern¹⁷. Homozygous recessive alleles at both loci result in the short day-long day requirements that underlie the univoltinism of *C. downesi*.

The preoviposition period of non-diapausing *C. downesi* is considerably longer than that of non-diapausing *C. carnea* (Table 1). The average preoviposition period of F₁ and F₂ hybrids and backcross progeny, reared in diapause-averting conditions is also consistently longer than that of *C. carnea*, and it is usually intermediate to those of *C. carnea* and *C. downesi* (Table 1). The range in the preoviposition period in the F₂ intercross progeny (see ranges on Table 1) shows very little overlap with the *C. carnea* range. This suggests that even one *C. downesi* allele at one of the two diapause-controlling loci can lengthen the preoviposition period. In addition, modifier genes could also be involved.

Genetics of adult coloration

To determine the mode of inheritance of the dark green adult coloration of *C. downesi*, we made reciprocal P₁ and F₁ crosses between *C. carnea* and *C. downesi*. We scored the colour of the F₁ and F₂ offspring using three broad categories: 'true *carnea*', 'true *downesi*', and 'intermediate'. We also divided the 'inter-

mediate' category into three subcategories: 'carnea-like', 'true intermediate', and 'downesi-like'.

All the F_1 offspring of the two reciprocal crosses of *C. carnea* $\times$ *C. downesi* fell into the 'intermediate' category; they ranged from 'carnea-like' to 'downesi-like' intermediates. In the F_2 progeny, coloration occurred in the three broad categories in ratios that do not differ from those expected for the segregation of a single pair of autosomal alleles—one recessive, one semidominant (Table 2). The 'intermediate' individuals again varied from 'carnea-like' to 'downesi-like'. Therefore, we conclude that *C. downesi* coloration is due primarily to a single, semidominant autosomal allele (which we designate as *G*); expressivity of the dark green colour is apparently modified by polygenes.

Sympatric speciation of *C. downesi*

On the basis of the distributional and biological²⁵ characteristics of *C. carnea* and *C. downesi*, we conclude that *C. downesi* is the more recently evolved. The multivoltine *C. carnea* occurs over most of the Holarctic Region, whereas the univoltine *C. downesi* is restricted to the more northern regions of North America. We know of no place where *C. downesi* is present without *C. carnea*, nor are we aware of evidence that the two species were ever geographically isolated.

Table 1 Preoviposition period of conspecific and interspecific crosses between *C. carnea* and *C. downesi*

Cross*		Preoviposition period (d)	
♀	♂	$\bar{x} \pm \text{s.d.}$ (no. of pairs)	Range
Intercross			
P_1			
d ¹ $\times$ d		8.5 $\pm$ 1.9 (6)	7–12
d $\times$ c		10.5 $\pm$ 4.5 (6)	7–19
c $\times$ d		12.6 $\pm$ 2.8 (5)	10–16
c $\times$ c		5.4 $\pm$ 0.6 (5)	5–6
F_1			
dd $\times$ dd		8.2 $\pm$ 0.97 (9)	7–10
dc $\times$ dc		5.3 $\pm$ 0.63 (13)	4–6
cd $\times$ cd		5.9 $\pm$ 0.88 (10)	5–7
cc $\times$ cc		4.3 $\pm$ 1.07 (10)	4–6
F_2			
dd-dd $\times$ dd-dd		13.0 $\pm$ 3.4 (9)	8–17
dc-dc $\times$ dc-dc		7.9 $\pm$ 2.6 (38)	5–19
cd-cd $\times$ cd-cd		9.4 $\pm$ 3.7 (13)	6–17
cc-cc $\times$ cc-cc		5.0 $\pm$ 0.66 (10)	4–6
Backcross			
P_1			
d $\times$ d		7.6 $\pm$ 1.7 (19)	6–12
d $\times$ c		9.3 $\pm$ 2.7 (6)	6–13
c $\times$ d		7.0 $\pm$ 3.0 (9)	4–13
c $\times$ c		5.3 $\pm$ 1.3 (7)	4–8
F_1			
dd $\times$ dd		12.3 $\pm$ 4.4 (37)	7–18
dc $\times$ dd		9.2 $\pm$ 1.8 (18)	5–12
dd $\times$ dc		13.7 $\pm$ 6.3 (13)	6–25
cd $\times$ dd		8.2 $\pm$ 1.7 (17)	6–12
dd $\times$ cd		13.9 $\pm$ 4.5 (12)	8–21
cc $\times$ cc		5.7 $\pm$ 1.6 (10)	4–9
F_2			
dd-dd $\times$ dd-dd		16.6 $\pm$ 4.2 (6)	10–20
dc-dd $\times$ dd-dd		11.0 $\pm$ 2.1 (16)	8–15
dd-dd $\times$ dc-dd		12.3 $\pm$ 3.1 (10)	8–16
cd-dd $\times$ dd-dd		14.4 $\pm$ 2.8 (17)	11–21
dd-dd $\times$ cd-dd		12.5 $\pm$ 2.6 (14)	9–17
dd-dc $\times$ dd-dd		10.2 $\pm$ 2.2 (12)	7–13
dd-dd $\times$ dc-dc		14.4 $\pm$ 2.9 (11)	9–19
dd-cd $\times$ dd-dd		13.2 $\pm$ 1.6 (17)	10–17
dd-dd $\times$ dd-cd		15.8 $\pm$ 3.1 (9)	12–20
cc-cc $\times$ cc-cc		5.8 $\pm$ 1.8 (9)	4–8

All experimental animals were reared under diapause-averting conditions (LD 10:14 until cocoon spinning, then LD 16:8; 24 $\pm$ 1 °C).

*d, *C. downesi*; c, *C. carnea*; ♀ parent always indicated first.

Table 2 Colour of F_2 adult progeny from crosses between *C. carnea* and *C. downesi*, compared with the expected values in a situation involving the inheritance of a semidominant allele

	'True carnea'	No. of adults 'True downesi'	Intermediate
Observed	78	65	146
Expected	72	72	145

$$\chi^2 = 1.25; P = 0.5-0.8.$$

We propose that *C. downesi* diverged sympatrically from an ancestral population of *C. carnea*, and that it did so through the establishment of a stable polymorphism for habitat utilisation and the subsequent development of reproductive barriers between the morphs in the two habitats.

The evolution of a stable polymorphism is based on the ancestral population's occurrence in two types of habitats: the meadow-field-deciduous tree habitat to which the population was well adapted, and the coniferous habitat, to which it was less well adapted. We propose that in the *C. downesi* ancestor the mutation of gene *g* to gene *G* (the autosomal, semidominant allele that produces the dark green cryptic coloration of *C. downesi* adults) provided a selective advantage for individuals carrying gene *G* in the coniferous habitat. The selective force that favoured gene *G* in this habitat was probably the action of vertebrate and invertebrate predators. This proposal is supported by our observations (in preparation) that conifer-inhabiting species of Chrysopidae are either cryptically (dark green) coloured (as in *Chrysopa harrisii* Banks and *C. downesi*) or they produce a malodorous secretion (as in *Meleoma emuncta* Banks) that is offensive to vertebrate and invertebrate predators²⁷. Therefore, in the coniferous habitat, the dark green colour of the *C. downesi* adult provided homozygous *GG* and heterozygous *Gg* adults with a selective advantage over the pale green (*gg*) form. Conversely, the light green, homozygous *gg* form retained its selective advantage over *G* individuals in the original habitat. The two homozygous forms, *GG* and *gg*, were therefore virtually restricted to separate habitats; whereas heterozygous *Gg* individuals, with their intermediate coloration, were at a competitive disadvantage in both habitats. Thus the population was subjected to disruptive selection. This selection presumably led to the establishment of a stable polymorphism involving light and dark green colour forms—each in its own habitat. Genetically-controlled preferential mating was not necessary to this step in the speciation process because disruptive selection maintained the balanced polymorphism even if both colour forms remained within a single random-mating population. But the frequency of pairings between *GG* and *gg* was probably low because of the restriction of adults of each colour form to their separate habitats.

The inheritance of *C. downesi*'s dark green colour is not unlike that proposed for early melanic forms of *Biston betularia* (L.). Kettlewell²⁸ deduced that the original *B. betularia* melanic form resulted from a single, semi-dominant allele which later became dominant. In contrast to the polymorphism in the ancestral *C. downesi*-*C. carnea* population, the balanced polymorphism in *B. betularia* is based on heterozygote superiority, a situation that would allow the evolution of dominance modifiers²⁹ and the maintenance of interbreeding between morphs. We propose colour change rather than alteration in habitat choice as the initial step leading to speciation of *C. downesi* because it allows the establishment of a stable, habitat polymorphism based on a single gene system. In contrast, alteration of habitat choice would have been more complex because at least two gene differences would be needed to establish a stable polymorphism.

Once a stable polymorphism was established, two selective forces probably favoured the evolution of the univoltinism of *C. downesi*, the characteristic that serves as the primary reproductive barrier between the forms. First, univoltinism

increased the adaptation of the coniferous form to its habitat, and second, it served to reduce or eliminate the wasteful production of the less fit heterozygotes. In contrast, the multivoltinism of the original form continued to be favoured in the original habitat. During summer, the number of conifer-inhabiting predators, such as other green lacewings (*C. harrisii*³⁰ and *M. emuncta*) and other arthropods (hemerobiids, syrphids, hemipterans, spiders, mites), increases, whereas available prey decreases (C.A.T. and M.J.T., in preparation). Therefore competition among conifer-inhabiting predators seems to be very intense during summer and we conclude that in the *C. downesi* ancestral form selection favoured a univoltine seasonal cycle that included reproduction in early spring, larval occurrence in mid to late spring, and reproductive diapause during summer, autumn and winter. This reproductive cycle would not have been advantageous to the original, non-coniferous form because in its habitat, prey were available throughout spring and summer.

Single allele differences at two loci underlie the seasonal asynchrony of *C. carnea* and *C. downesi*¹⁷. Thus, the mutation of the recessive 'downesi' alleles at these two loci and their replacement of the dominant 'carnea' alleles in the dark green coniferous form, resulted in the evolution of the seasonal reproductive barrier between *C. carnea* and *C. downesi*. We represent the mutation of the two diapause-controlling alleles from 'carnea' to 'downesi' as $D_1 \rightarrow d_1$ and $D_2 \rightarrow d_2$.

The evolution of the unicyclic *C. downesi* seasonal cycle probably took place in two separate phases: (1) the addition of the aestival diapause and (2) the extension of the autumnal diapause through winter. The summer diapause conceivably reduced the incidence of interform pairings by approximately two-thirds, because two of *C. carnea*'s three annual generations reproduce during summer. The extension of the autumnal diapause through winter probably eliminated the interbreeding of overwintering populations by delaying sexual maturation in *C. downesi* in relation to *C. carnea*. We propose that each of the two diapause-controlling loci regulates one of these two differences in the seasonal cycles. Recessive 'downesi' alleles (d_1d_1) at one locus probably result in diapause induction in long-day conditions, and this locus functions in the expression of the aestival diapause. Recessive 'downesi' alleles (d_2d_2) at the second locus probably act in maintaining diapause until after short days are experienced and they therefore serve to prolong diapause through winter. We have no evidence showing which of the alleles (d_1 or d_2) first mutated and/or replaced its dominant counterpart in the coniferous form. The 'downesi' alleles were strongly selected against in the original non-coniferous form because of their negative effect on the reproduction rate. Even one d_1 or d_2 allele at one of the diapause-controlling loci lengthens the preoviposition period (Fig. 1), which, in the original habitat would be clearly deleterious. Thus, strong disruptive selection resulted in the replacement of the dominant 'carnea' alleles by the recessive 'downesi' alleles in the coniferous form. As a result, the seasonal cycles of the two forms became asynchronous, interbreeding between the forms ceased, and *C. downesi* was established as a species.

Subsequent to or concurrent with the evolution of reproductive isolation, at least two types of changes increased *C. downesi*'s fitness in the coniferous habitat: (1) the evolution of habitat selection and (2) modification of the thermal requirements for development. Those *C. downesi* individuals that recognise and remain in the coniferous habitat would have a higher chance of leaving offspring than those that choose the non-coniferous habitat or that choose their habitat at random. This behaviour is probably genetically controlled, but the numbers and types of genes involved are unknown.

The second change in *C. downesi* was the alteration in the lower thermal threshold (t) for growth and development. *C. downesi* from Ithaca has a slightly ($\sim 2^\circ\text{C}$) but significantly higher t value than *C. carnea* from the same area¹⁹. In nature, this slightly higher threshold acts as a precise fine-tuner of *C. downesi* univoltinism.

Genetic model

Using our data from *C. downesi*, we present a genetic model for sympatric speciation through habitat diversification and seasonal isolation by non host-specific animals. Our model begins with a hypothetical ancestral species that is well-adapted to several habitats. This species is neither restricted to a particular host, nor confined to a particular plant association. Occasionally it occurs in a secondary habitat to which it is less well adapted. Also, it is multivoltine and reproduction encompasses a broad period of the annual cycle.

Step 1, the establishment of a stable polymorphism in a two-habitat situation, is similar to the first step in Maynard Smith's general model⁷ for sympatric speciation. It begins with the mutation of gene a to gene A , a semi-dominant allele that confers a selective advantage on homozygous AA individuals in the secondary habitat. Disruptive selection acts in favour of aa individuals in the original habitat, in favour of AA individuals in the secondary habitat, and against Aa individuals in both habitats. Thus, a balanced polymorphism involving the A and a alleles that adapt individuals to differing habitats is established even if both morphs remain in a single, random-mating population. Assortative mating, if it does occur in the two forms because of their habitat differences, would probably not have a genetic basis.

Step 2, the evolution of seasonal isolation, involves the non-simultaneous mutation and selection of diapause-controlling alleles that restrict the seasonal period of reproduction in the new form. In our model, recessive alleles (b and c) at two unlinked loci act individually or in combination to produce a restricted (or unicyclic) seasonal cycle. Selection pressure for the b and c alleles in the new morph comes from two sources: (1) by synchronising reproduction with favourable seasonal conditions in the new habitat, the alleles increase the adaptation of the new AA form, and (2) by reducing the incidence of interform pairings they reduce the production of the less fit Aa heterozygotes. Because the original, multivoltine seasonal cycle is well adapted to the original habitat, selection acts to keep the recessive b and c alleles out of the aa population; this selection is enhanced if the diapause-controlling alleles in the heterozygous condition also produce unfavourable effects. Disruptive selection such as this leads to the establishment of two seasonally-isolated populations—the original $aaBBCC$ in the original habitat and the new $AAbbcc$ in the new habitat. With the completion of this process, the new species is established.

Step 3 in our model comprises changes that increase the fitness of the newly-formed species. For example, selection favours individuals of the new species that recognise and choose the 'correct' habitat. Therefore, genetic changes associated with the physiology and behaviour of habitat selection are likely to occur. Selection also favours changes that fine-tune the seasonal isolation of the two species and that increase the new species' synchrony with favourable seasonal conditions in its habitat. The physiological characteristics involved in these changes are usually under polygenic control^{26,31,32}.

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- ¹ Mayr, E. *Animal Species and Evolution* (Belknap, Cambridge, Massachusetts, 1963).
- ² Wallace, B. *Topics in Population Genetics*. (W. W. Norton, New York, 1968).
- ³ Alexander, R. D. *Q. Rev. Biol.* **43**, 1–41 (1968).
- ⁴ Thoday, J. M. *Proc. R. Soc. B* **182**, 109–143 (1972).
- ⁵ Bush, G. L. *A. Rev. Ecol. Sys.* **6**, 339–364 (1975).
- ⁶ Ayala, F. J. in *Evolutionary Biology* (eds Dobzhansky, T., Hecht, M. K. & Steere, W. C.), 1–78 (Plenum, New York, 1975).
- ⁷ Maynard Smith, J. *Amer. Nat.* **100**, 637–650 (1966).
- ⁸ Dickinson, H. & Antonovics, J. *Am. Nat.* **107**, 256–274 (1973).
- ⁹ Bush, G. L. *Evolution* **23**, 237–251 (1969).
- ¹⁰ Kerner, G. & Atwood, C. E. *Science* **179**, 1090–1099 (1973).
- ¹¹ Phillips, P. A. & Barnes, M. M. *Ann. ent. Soc. Amer.* **68**, 1053–1060 (1975).
- ¹² Khasimuddin, S. & DeBach, P. *Entomophaga* **21**, 113–122 (1976).
- ¹³ Bush, G. L. in *Evolutionary Strategies of Parasitic Insects and Mites* (ed. Price, P. W.), 187–206 (Plenum, New York, 1975).
- ¹⁴ Mayr, E. *Sys. Zool.* **12**, 204–206 (1963).

- 15 Alexander, R. D. *Syst. Zool.* 12, 202–204 (1963).
 16 Masaki, S. *Bull. Fac. Agric. Hirosaki Univ.* 11, 59–90 (1965).
 17 Tauber, C. A., Tauber, M. J. & Nechols, J. R. *Science* (in the press).
 18 Tauber, C. A. & Tauber, M. J. *Science* (in the press).
 19 Tauber, M. J. & Tauber, C. A. *Can. J. Zool.* 54, 260–265 (1976).
 20 Zeleny, J. *Acta ent. bohemoslov.* 62, 177–194 (1965).
 21 Sheldon, J. K. & MacLeod, E. G. *Trans. Am. ent. Soc.* 100, 437–512 (1974).
 22 Tauber, M. J. & Tauber, C. A. *Nature* 244, 296–297 (1973).
 23 Tauber, M. J. & Tauber, C. A. *J. Insect Physiol.* 19, 1455–1463 (1973).
 24 Tauber, M. J. & Tauber, C. A. *Nature* 258, 711–712 (1975).
 25 Tauber, M. J. & Tauber, C. A. *J. Insect Physiol.* 22, 331–335 (1976).
 26 Tauber, M. J. & Tauber, C. A. in *Evolution of Escape in Space and Time* (ed. Dingle, H.) (Springer, Berlin, 1977).
 27 Blum, M. S., Wallace, J. B. & Fales, H. M. *Insect Biochem.* 3, 353–357 (1973).
 28 Kettlewell, H. B. D. *A. Rev. Ent.* 6, 245–262 (1961).
 29 Haldane, J. B. S. *Proc. R. Soc.* 145, 303–306 (1956).
 30 Tauber, M. J. & Tauber, C. A. *Can. Ent.* 106, 969–978 (1974).
 31 Dingle, H. in *Experimental Analyses of Insect Behavior* (ed. Barton Brown, L.), 331–342 (Springer, Berlin, 1974).
 32 Istock, C. A., Zisfein, J. & Vavra, K. *J. Evolution* 30, 535–547 (1976).

letters to nature

Variable mid-latitude X-ray source 3U0042+32

THE faint high latitude X-ray sources are a mixed population of galactic and extragalactic objects. Identified extragalactic X-ray sources include clusters of galaxies, Seyfert and other active galaxies, quasars, and nearby normal galaxies^{1,2}. Identified galactic sources with $|b^{\text{II}}| > 20^\circ$ include several X-ray binaries, two in globular clusters—M15 (ref. 1) and NGC1851 (ref. 3)—the hot white dwarf HZ43 (ref. 4), the dwarf binary AM Her (ref. 5) and the flare star UV Ceti (ref. 6). The high-latitude sources generally lie in fields of low obscuration and density of objects so that the prospects for their identification with optical or other counterparts are relatively good, given position measurements of moderate accuracy. We report here a precise celestial location (1' error radius) for one such X-ray source, 3U0042+32 (ref. 1), that is situated at mid-galactic latitude ($b^{\text{II}} \sim -30^\circ$). A study of the error box for this source indicates that it may be a distant galactic binary system similar to Her X-1.

In early February 1977, Ricketts and Cooke⁷ reported a flareup in the intensity of a source in the region of 3U0042+32. Their observations, carried out with the Ariel V X-ray satellite, showed that this source, which had been below the level of detectability during a total of 50 d of scanning since November 1974, was variable up to 25 Uhuru counts per s during 3–6 February, 1977 and up to 50 Uhuru counts per s during 14–17 February. Variability in 3U0042+32 had previously been reported during the interval 1971–73 (refs 8 and 9).

Following receipt of a private communication of the Ariel-5 observation, the SAS-3 X-ray observatory was oriented to bring the flaring source within the field of its rotation modulation collimators. At the time, the location of the Sun was such as to restrict the period of useful star tracker data to only 20 min per orbit. Nevertheless, sufficient exposure was obtained during 25–26 February, 1977 to enable us to make position measurements with the data from both the 2.3 and 4.5 arc minute collimators and in two separate energy channels.

The characteristics and use of the rotation modulation collimator detectors on SAS-3 have been described previously by Doxsey *et al.*¹⁰ and Schnopper *et al.*¹¹.

Table 1 summarises the results on the position derived from the two independent modulation collimators. For each collimator the data were subdivided into two parts, representing the first and second halves of the observation. The mean position obtained from the combined results is $\alpha = 00^{\text{h}} 42^{\text{m}} 08.5^{\text{s}}$; $\delta = 32^\circ 44' 53''$ (epoch 1950.0) with an error circle of 1' radius (90% confidence).

Figure 1 is an enlargement of the red Palomar Sky Survey print of this region with the SAS-3 error circle superposed on it. Also shown are the error box of 3U0042+32, the intensity of which is listed as 7.0 ± 0.5 counts per s in the Uhuru catalogue¹, and the error box of the flaring source studied with Ariel V. It is likely that all three observations pertain to the same object, and that it lies inside the SAS-3 error circle. Within or very near this circle, there are seven stellar images above the plate limit and with visual magnitude $\lesssim 15.5$. Harris *et al.*¹² have studied this region in detail at both radio and optical wavelengths. They point out only one weak radio source in the

Fig. 1 Enlargement of the red Palomar Sky Survey print of the region near 3U0042+32 (copyright National Geographic Society). The solid lines represent portions of the Uhuru (3U) and Ariel V error boxes^{1,7} for this source. The 1 min radius error circle is derived from data obtained with the rotation modulation collimators on SAS-3 (see text).

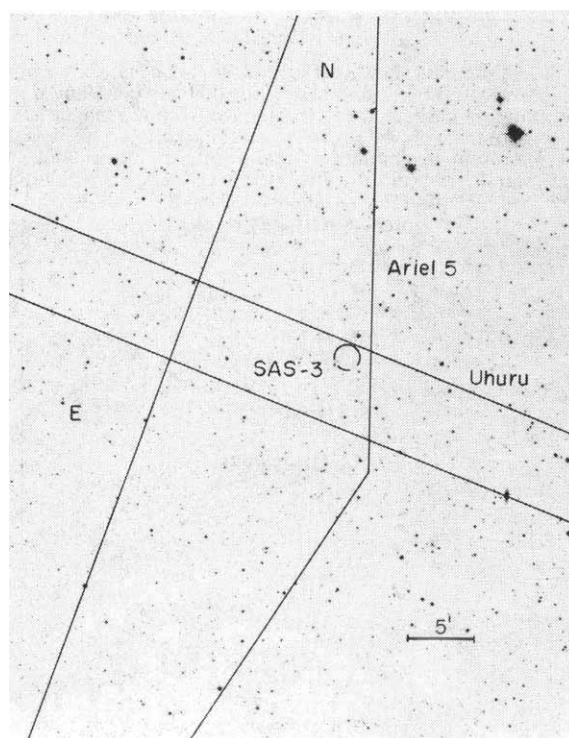


Table 1 Rotating modulation collimator positions* for 3U0042+32

	2.3' FWHM collimator	4.5' FWHM collimator
Data set 1	$\alpha = 00^{\text{h}}42^{\text{m}}10^{\text{s}}$ $\delta = 32^\circ 45' 06''$	$\alpha = 00^{\text{h}}42^{\text{m}}06^{\text{s}}$ $\delta = 32^\circ 44' 45''$
Data set 2	$\alpha = 00^{\text{h}}42^{\text{m}}09^{\text{s}}$ $\delta = 32^\circ 44' 57''$	$\alpha = 00^{\text{h}}42^{\text{m}}09^{\text{s}}$ $\delta = 32^\circ 44' 43''$
Average	$\alpha = 00^{\text{h}} 42^{\text{m}} 08.5^{\text{s}}$ $\delta = 32^\circ 44' 53''$	

*Epoch 1950.0

vicinity of the SAS-3 error box ($\sim 2.2'$ from the centre). Bahcall *et al.*¹³ have also searched for the optical counterpart to this source, but without success.

At the conclusion of the position measurement SAS-3 was switched to the fast timing mode (8 ms resolution; see ref. 14). The source was studied for regular pulsations in the range 16 ms to 20 s. Analysis of data from three satellite orbits yielded an upper limit to pulsing in this range of $\sim 15\%$ of the steady source intensity (~ 35 Uhuru counts per s). During 27.3–27.7 February, 1977, the y -axis detectors¹⁵ were pointed at the source in order to search for possible longer period pulsations. These results also proved negative with a pulsing limit of $\sim 7\%$ set in the range of 1 s to ~ 500 s.

Spectral data in the energy range 1–19 keV were also obtained with the y -axis detectors. The energy spectrum is nearly flat out to ~ 10 keV ($\alpha = 0.0 \pm 0.4$), with a characteristic cutoff energy of ≤ 1.5 keV. At higher energies the spectrum falls off rapidly with increasing energy.

The peak X-ray flux from this source (50 Uhuru counts per s) is ~ 50 times greater than the optical flux from the brightest object ($m_v \approx 15.5$) in the error circle at the time of the Palomar exposure. The timescale of its variations, according to the Ariel V observations, is of the order of days. Its spectrum resembles that of hard X-ray binaries which derive their power from accretion on to a compact star. We believe that the most likely explanation of this flaring source is that it is a neutron star in a close binary system with a companion that undergoes occasional episodes of mass transfer due to eruption or orbital eccentricity (compare ref. 16).

The limit on the optical magnitude of the stellar companion ($m_v \geq 15.5$) places significant constraints on the distance to 3U0042+32 for various assumed spectral types. These distance constraints are summarised in Fig. 2. For each spectral type the absolute visual magnitudes of a main sequence dwarf and subdwarf star¹⁷ were used; any larger stars would require still larger distances. A companion star earlier than spectral type B5 would probably lie beyond the galactic halo. Stars of very early type are likely to be excluded, in any case, because a comparison of the Palomar red and blue plates indicates that none of the stellar images within the SAS-3 error circle exhibits a large blue excess. Only stars of type $\sim K5$ or later would be consistent with a location within ~ 200 pc of the galactic plane. A distant late A or early F star, similar to HZ Her^{18,19}

(= Her X-1) would be consistent with all presently available data on 3U0042+32.

The possibility that the source is extragalactic is remote. The peak X-ray intensity of the source is equal to that of the Perseus Cluster¹ yet there is no active galaxy or known quasar in the error circle^{12,13}. The short timescale of the X-ray variations implies that the source must be 'compact' (that is, $\lesssim 10^{16}$ cm) as opposed to, for example, an extensive region of hot intra-cluster gas. One might then expect the spectrum of such an extragalactic object to be highly absorbed, by analogy with other compact extragalactic sources (for example, Cen A, 3C273, and NG4151; ref. 20). On this latter point, our limited spectral data may not be decisively negative. Nevertheless, if the source is extragalactic, it must be of a kind not previously observed.

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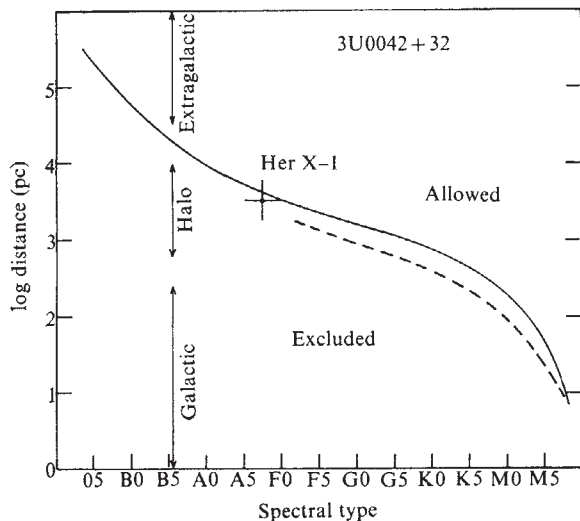
S. RAPPAPORT
G. W. CLARK
R. DOWER
R. DOXSEY
G. JERNIGAN
F. LI

Department of Physics
and Center for Space Research,
Massachusetts Institute of Technology,
Cambridge, Massachusetts 02139

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- 1 Giacconi, R. *et al. Astrophys. J.*, suppl., **27**, 37–64 (1974).
- 2 Cooke, B. A. *et al. M. Not. R. astron. Soc.* (in the press).
- 3 Clark, G. W., Markert, T. H. & Li, F. K. *Astrophys. J. Lett.* **199**, L93–L96 (1975).
- 4 Hearn, D. R. *et al. Astrophys. J. Lett.* **203**, L21–L24 (1976).
- 5 Hearn, D., Richardson, J. & Clark, G. W. *Astrophys. J. Lett.* **210**, L23–L26 (1976).
- 6 Heise, J. *et al. Astrophys. J. Lett.* **202**, L73–L76 (1975).
- 7 Ricketts, M. J. & Cooke, B. A. *IAU Circ. No.* 3039 (1977).
- 8 Markert, T. M. *et al. Astrophys. J.* **206**, 265–272 (1976).
- 9 Murray, S. S. & Ulmer, M. P. *Astrophys. J.* **210**, 230–232 (1976).
- 10 Doxsey, R. *et al. Astrophys. J. Lett.* **203**, L9–L12 (1976).
- 11 Schnopper, H. W. *et al. Astrophys. J. Lett.* **210**, L75–L78 (1976).
- 12 Harris, D. E., Bahcall, N. A. & Strom, R. G. *Astron. Astrophys.* (in the press).
- 13 Bahcall, J. N., Bahcall, N. A., Murray, S. S. & Schmidt, M. *Astrophys. J. Lett.* **199**, L9–L11 (1975).
- 14 Primini, F. *et al. Astrophys. J. Lett.* **210**, L71–L74 (1976).
- 15 Lewin, W. H. G. *et al. M. Not. R. astron. Soc.* **177**, 93P–100P (1976).
- 16 Bradt, H. *et al. Astrophys. J. Lett.* **204**, L67–L71 (1976); and references therein.
- 17 Allen, C. W. *Astrophysical Quantities* 3rd edn (Athlone, London, 1973).
- 18 Crampton, D. *Astrophys. J.* **187**, 345–348 (1974).
- 19 Bahcall, J., Joss, P. C. & Avni, Y. *Astrophys. J.* **191**, 211–219 (1974).
- 20 Kellogg, E. M. *IAU Symp. No. 55* (eds Bradt, H. & Giacconi, R.) 171–183 (Reidel, Dordrecht, 1973).

Fig. 2 Minimum allowed distance to 3U0042+32 against assumed spectral type of an hypothetical binary companion star. The solid and dashed curves are derived from the magnitude of the brightest star in the SAS-3 error circle ($m_v \sim 15.5$) and the absolute visual magnitudes of main sequence dwarf and subdwarf stars¹⁷, respectively. Interstellar extinction was assumed to be negligible. Parameter values for Her X-1 (refs 18 and 19) are shown for reference.



Supernovae and molecular clouds

In a study of the CO distribution in the Galaxy, Bash and Peters¹ concluded that there is an upper limit of 30 Myr to the age of stellar systems which contain CO. Younger systems, launched by the impulse of the density wave show abundant CO emission. Systems which have existed for longer than 30 Myr display no CO emission. Based on their initial observations, Bash and Peters predicted that there would be a correlation between the presence of CO emission and the turnoff in open clusters. Observations by Green confirm this prediction² showing CO emission in at least 24 of 28 clusters with turnoff earlier than spectral type B0 and no evidence of CO emission in 35 of 38 clusters with turnoff later than B0. We suggest that there is a critical mass M_{crit} such that for $M > M_{\text{crit}}$ stars do not evolve to an explosive phase and hence the surrounding molecular cloud is substantially unaffected by the evolving stars. As the system ages, the most massive star with $M < M_{\text{crit}}$ finally evolves to an explosive endpoint and the resulting supernova dissipates the dense molecular cloud. From the cluster observations we identify $M_{\text{crit}} \sim 15\text{--}20 M_{\odot}$, the mass of a B0 star (ref. 3 and E. Green,

private communication), although there is some uncertainty in luminosity class.

An alternative is that one is merely seeing an excitation phenomenon, that stars with $M > M_{\text{crit}}$ are necessary to excite the CO molecules and those with $M < M_{\text{crit}}$ are unable to excite. The basic argument against this possibility is that the disappearance of CO emission seems to be correlated with the disappearance of HII regions as well². HII and CO regions have very different excitation characteristics and, hence it seems unlikely that they could both be made to vanish simultaneously by altering a single source of excitation.

A crucial point to the present argument is that a supernova be able to disrupt the molecular cloud. Molecular clouds have mass up to $\sim 10^5 M_{\odot}$ and density $\sim 10^4 \text{ cm}^{-3}$. Such large clouds have a gravitational binding energy of $\sim 2 \times 10^{50}$ erg and an atomic/molecular binding energy $\sim 3 \times 10^{51}$ erg. Thus, a healthy supernova should be able to disrupt a cloud as massive as this if all its energy is utilised for this purpose. Mouschovias⁴ argues, however, that a supernova in such a cloud will pass from the adiabatic Sedov phase to the isothermal, or snowplow, phase long before the shock produced by the supernova reaches the surface of the cloud. If this were the case only the momentum at the onset of the isothermal phase, not the energy of the supernova, would be available. The initial momentum of a typical supernova, $(2M_{\text{SN}}E_{\text{SN}})^{1/2}$, is capable of giving an outward velocity of $v \sim 3.2 \text{ km s}^{-1} ((M_{\text{SN}}/10M_{\odot})^{1/2} E_{51})^{1/2} M_{\text{cl},4}^{-1}$. The escape velocity is $v_{\text{es}} \sim 4.5 \text{ km s}^{-1} (M_{\text{cl},4})^{1/3} n_4^{1/6}$. Thus, a $10^4 M_{\odot}$ cloud can be disrupted by a supernova of $\sim 20 M_{\odot}$, roughly the mass we are considering.

Molecular clouds, like stars, follow a fairly steeply decreasing mass function. While the details are not fully agreed on, one can argue that a typical cloud probably contains of order $10^4 M_{\odot}$; that the $10^5 M_{\odot}$ clouds are observationally striking, but not representative. In particular, $10^4 M_{\odot}$ is probably a more typical mass for the molecular clouds surrounding the young open clusters with which we are especially concerned. Thus we conclude that supernovae are the likely cause of disruption of most molecular clouds, even if only their momentum, not their full energy is utilised.

For a $10^4 M_{\odot}$ cloud with uniform density of 10^4 cm^{-3} the radius (R) is 2.2 pc, the dissociation energy is $\sim 3 \times 10^{50}$ erg and the binding energy $\sim 4 \times 10^{48}$ erg. A supernova of $10M_{\odot}$ and 10^{51} erg will sweep up its own mass entering the Sedov phase at $R \sim 0.2$ pc and $t \sim 30$ yr. This adiabatic phase will end at ~ 0.5 pc and ~ 300 yr when the radiation cooling time drops below the expansion timescale. The rapid leakage of radiation will begin at temperature, $T \sim 1 \times 10^7$ K for these high densities and be further enhanced at $T \sim 10^6$ K when recombination radiation from heavy ions becomes very strong. An HII region will then race ahead of the original shock front dissociating the cloud. This will lead to a loss of thermal energy and eventually the ionisation energy as the cloud expands and recombines. For a $10^5 M_{\odot}$ cloud the HII region is probably unable to totally dissociate the cloud. On recombination, the Lyman α flux will be re-radiated from grains as a burst of $10 \mu\text{m}$ radiation which will escape from the cloud.

Thus while the details are different because of the dense cloud, the evolution of the explosion may be qualitatively similar to the standard picture⁵ and we conclude that Mouschovias' arguments concerning the energetics are basically correct. A more careful treatment would incorporate possible density gradients in the cloud. A decreasing density outward, as is observed in many clouds, might serve to prolong the Sedov phase. A step function from low to high density due to a pre-existing HII region would lead to reflected shocks and perhaps an elimination of the already rather brief Sedov phase. These details are presently under consideration in a program which will lead to detailed numerical calculations.

In the simplest picture, the longest lived very bright phase, and hence the one most likely to be observed, may be the Sedov phase. There is an appreciable flux of radiation in this phase, primarily thermal bremsstrahlung, even though it is not sufficient to effect the shock dynamics. This flux of soft X rays will probably be absorbed on grains, raising them to several hundred K and again will be seen in the $10 \mu\text{m}$ range. The estimated flux is $\sim 5 \times 10^6 L_{\odot}$ for a time of

about 100 yr. If supernovae occur in our Galaxy with a mean time interval $\lesssim 40$ yr then the present number of events could be $\gtrsim 3$ if they each last ~ 100 yr. Kwan and Scoville⁶ have suggested that the Becklin-Neugebauer object may be the site of an explosion.

We have suggested in this note that there exists an upper limit to the mass of stars which explode, $\sim 15\text{--}20M_{\odot}$. This upper limit might exist because of differences in internal structure at the state just before iron core collapse in the final stages of evolution. This possibility leads to the suggestion that stars earlier than BO do not explode because they collapse to make black holes, a notion which is consistent with a suggestion made previously by Wheeler and Shields⁷. Alternatively, these massive stars are known to lose mass and the possibility exists that their mass is reduced to that of a BO before significant evolution from the main sequence can occur (T. Mazurek, personal communication). In this case, the changes in the internal structure, if any, are unclear.

In either case, if this conjecture about an upper mass limit to supernovae proves to be true, quantitative, but perhaps not qualitative, changes might have to be made in notions of nucleosynthesis. Stars from, say, $10M_{\odot}$ to $20M_{\odot}$ would still be available as sites of explosive nucleosynthesis and, because of the steep mass function, these are most of the stars which would contribute anyway.

Another important ramification of this suggestion is that star formation would cease with the supernova explosion and the dissolution of the cloud. This picture thus accounts very naturally for the observed inefficiency of star formation in molecular clouds.

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J. CRAIG WHEELER
FRANK N. BASH

Department of Astronomy
The University of Texas at Austin,
Austin, Texas 78712

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- ¹ Bash, F. N. & Peters, W. L. *Astrophys. J.* **205**, 786 (1976).
- ² Bash, F. N., Green, E. M. & Peters, W. L. *Astrophys. J.* in press (1977).
- ³ Ostriker, J. P., Richstone, D. O. & Thuan, T. X. *Astrophys. J. Lett.* **188**, L87 (1974).
- ⁴ Mouschovias, T. Ch. *Astrophys. J.* **207**, 141 (1976).
- ⁵ Spitzer, L. Jr. *Diffuse Matter in Space* (Interscience, New York, 1968).
- ⁶ Kwan, J. & Scoville, N. *Astrophys. J. Lett.* **210**, L39 (1976).
- ⁷ Wheeler, J. C. & Shields, G. A. *Nature* **259**, 642 (1976).

Molecular images by electron-wave holography

IMAGES of electron clouds in gas-phase atoms have been photographed^{1,2} at a resolution limit of $0.1 \text{ \AA}$ through a two-stage holographic microscope based on Gabor's principle of image reconstruction³. Information encoded on to holograms with electron radiation in the first stage was transformed into a portrayal of electron density in the second stage with the aid of an optical laser. Suitable pictures of gas-phase molecules could not be obtained because of signal-to-noise problems. These problems have now been overcome to a considerable extent and we report here the reconstruction of spherically averaged molecular images displaying light atoms in the vicinity of heavy atoms. Bond lengths can easily be measured with a ruler.

To make holograms yielding high resolving power, it is necessary to produce a broad reference wave that can be mixed coherently with waves emanating from the object under scrutiny. Stringent mechanical stability is also essential. Optical path length differences between the reference beam (source to detector) and object waves (source to object to detector) must not vary during recording by more than a fraction of a wavelength. To achieve these requirements in optical holography, great care must be taken. To achieve them with electron holography, where wavelengths are only one $1/100,000$ as long, would be virtually impossible unless the reference beam diffuser were locked internally in the molecular system to be

viewed. In atomic holography this was accomplished very naturally for electron cloud objects by scattering the incident wave from the atomic nuclei associated with the electron clouds. In this study of molecules a heavy atom in each molecule served to scatter a strong reference beam. In both cases, then, each particle in the sample to be investigated carries around its own reference wave diffuser, as it were, and produces its own hologram. Holograms from all particles in the sample fall on top of each other. Therefore, the reconstructed images also all fall on top of each other. Instead of seeing individual atoms or molecules we see the average of an ensemble. If all members are identical the images are not degraded. Because our molecular objects are in the vapour phase to reduce extraneous scattering, they have chaotic orientations. Accordingly, we see only the spherical average. This might seem disappointing, but it should be noted that any individual, free molecule in any given quantum state is inescapably diffuse in orientation, and spherically delocalised in the ground quantum state.

For our first molecular investigation we chose AsF_5 , an example where five light 'object' atoms (fluorine) are packed with D_{3h} symmetry around a central heavy atom (arsenic). What we should expect to see and do in fact see in the reconstructed image is a spherical shell of atomic fluorines. The radius of this shell corresponds to the mean As-F bond length.

For the first stage of our holographic microscope we used the apparatus previously used for atoms², but we reduced the numerical aperture twofold, to $f/2.3$. Resolving power was sacrificed to record with maximum faithfulness the strongest holographic interference fringes. Holograms were recorded on Process plates by scattering 40-kV electrons ($\lambda = 0.06 \text{ \AA}$) from a fine jet of AsF_5 vapour at 20 torr and filtering the electrons through an r^3 rotating sector. This filter is deliberately weaker than the 'Rutherford filter'² adopted for monatomic samples to bring out the detail of the light atoms more strongly. Once obtained, the holograms were reduced 18-fold by rephotographing on Kodak 120-02 holographic plates.

Rendering the light atoms visible in the presence of the heavy central atom required a new design for the second, reconstruction stage of the microscope. As before, the desired image is a component of the Fraunhofer diffraction pattern of the hologram. But the holographic fringes corresponding to the As-F bonds are comparatively subtle, and the reconstructed image of fluorine atoms lies very close to the intense 'undiffracted' zero-order beam transmitted by the hologram. (The radius of the molecular image occurs at about the tenth ring of the Airy diffraction pattern produced by the holographic aperture. The beam stop illustrated in Fig. 1 screens through the third Airy ring.) If left at its natural level, this zero-order beam overwhelms the desired image. In principle, the most efficient way of getting rid of the unwanted beam without distorting the molecular image would be to cancel it with another, properly modulated, beam in a Mach-Zehnder interferometer². In practice, it proved much simpler and adequately effective to use a technique suggested by Professor Emmett Leith, namely to remove the zero-order beam differentially by the 'spatial domain filter'⁴ illustrated in Fig. 1. An internal calibration of the

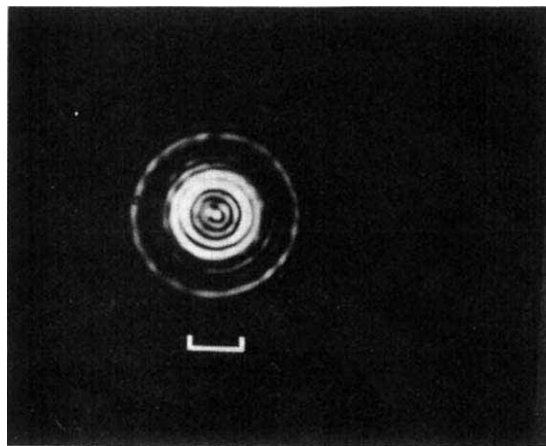


Fig. 2 Holographic image of rotational average of AsF_5 molecules at 70 million power; spherical shell of fluorine atom density plainly visible at $1.6 \text{ \AA}$ radius. Scale bar $1 \text{ \AA}$.

magnification afforded by the second stage of the microscope could be made easily by measuring the interference spots produced by a 100 line per inch Ronchi grating placed over the hologram.

A reproduction of an image of the rotationally averaged AsF_5 molecule at an Abbe resolution limit of $0.2 \text{ \AA}$ is shown in Fig. 2. Because of the curvature of the spherical shell of fluorines, the maximum density of the projected image lies a few hundredths of an angstrom unit inside the actual mean bond length of $1.68 \text{ \AA}$ (ref. 5). In principle it would be possible to deconvolute the projected density in Fig. 2 and determine the amplitude of vibration of the As-F bonds from the breadth of the fluorine shell, but this has not yet been done. Since the computed² depth of focus of our microscope at the numerical aperture used is about $4 \text{ \AA}$ and, hence, is appreciably greater than the As-F distance, the three-dimensional aspect of the spherical image is largely lost. As is the case in conventional holography there are 'extraneous contributions' to the image arising from incoherent scattering and from the square of the object amplitude. In heavy exposures the latter contributions show up as shells revealing the $\text{F} \cdots \text{F}$ non-bonded distance occurring at $\sqrt{2} r_{\text{AsF}}$, and vestiges of the non-bonded distance at about $\sqrt{3} r_{\text{AsF}}$. Indeed, given a molecule without a single dominant heavy atom, the laser reconstruction would yield series of concentric shells the profile of which would be closely related to the radial distribution function of internuclear distances in the molecule.

For many years electron diffraction patterns have been measured with microdensitometers sensitive to changes in absorbance of a few parts per ten thousand. Molecular structures have then been deduced by computer analysis of diffracted intensities. In our technique, a much more direct display of structural information is provided immediately by the optical Fourier spectrum of the electron hologram. Although the new technique is not yet competitive in accuracy with the well established scattering techniques, it may find a useful role. Whether analogous techniques can be worked out to study oriented molecules is a challenging problem for the future.

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L. S. BARTELL
R. D. JOHNSON

Department of Chemistry,
University of Michigan,
Ann Arbor, Michigan 48109

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¹ Bartell, L. S. & Ritz, C. L. *Science* **185**, 1163 (1974).

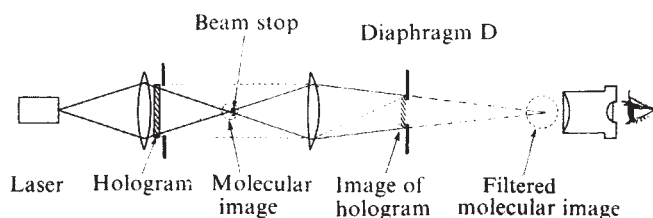
² Bartell, L. S. *Optik* **43**, 373 (1975); **43**, 403 (1975).

³ Gabor, D. *Nature* **161**, 777 (1948); *Proc. R. Soc. A* **197**, 454 (1949).

⁴ Leith, E. N. *Photogr. Sci. Engng* **6**, 75 (1962).

⁵ Clippard, F. B. & Bartell, L. S. *Inorg. Chem.* **9**, 805 (1970).

Fig. 1 Schematic drawing of optical reconstruction stage of holographic microscope. The unwanted beam passing undiffracted through the holographic fringes is largely screened out by the beam stop; most of the remainder is skimmed off by diaphragm D which passes the image-forming rays.



Formation of silver mirror by photolysis of thin films of high molecular weight silver compounds

SILVER salts of carboxylic acids such as formic acid, acetic acid and oxalic acid¹ became coloured in light. Similarly, suspensions of silver polyalkylacrylates produce various coloured colloidal solutions of silver in light². These phenomena show that silver carboxylates are easily photolysed to separate silver. We have investigated the photolyses of silver salts of certain high molecular carboxylic acids such as polyacrylic acid, polymethacrylic acid, poly-L-glutamic acid³, alginic acid⁴, pectic acid⁵, carboxymethylcellulose and cellulose acetate phthalate, and we report that the smooth films of these silver salts form a silver mirror arising from photolytic silver on their surfaces. This method of producing a thin silver film on the polymer film may be of technical interest because of the optical and electrical properties of the irradiated film.

Thin films of high molecular weight silver carboxylates (about 4 μm thick) were prepared in the following way. The aqueous solutions of the sodium salts of about 0.5–1% (1 ml) were coated on 20-cm² glass plates and dried in the air, then the plates were immersed in silver nitrate solution of 0.1 M for 1 h and washed with water to eliminate sodium nitrate and excess silver nitrate contained in the films, and dried again in the air. For preparation of films of silver salts with smooth surfaces it was essential that the aqueous solutions of sodium salts were dried sufficiently to form the films of sodium salts on the glass plates. This technique thoroughly converted the films of soluble sodium salts into films of slightly soluble silver salts by a rapid ion exchange reaction between sodium ions and silver ions in the films.

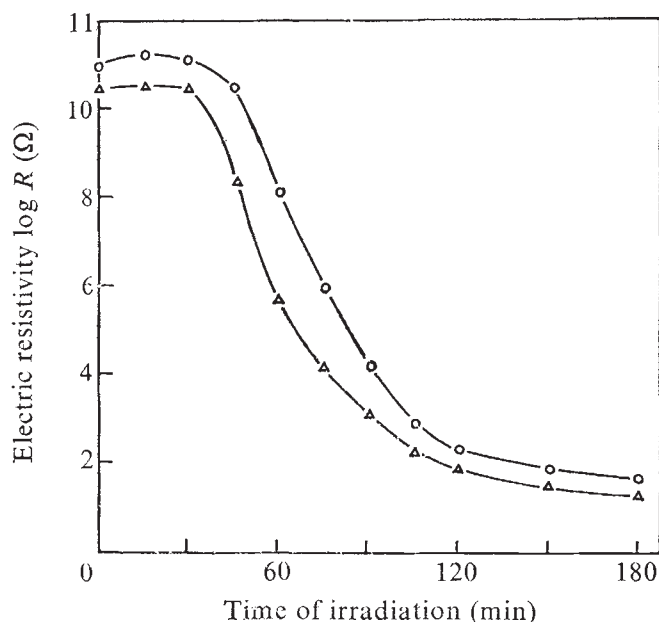


Fig. 1 Plot of log resistivity of the irradiated surface against time of irradiation for silver polyacrylate (O) and silver carboxymethylcellulose (Δ). The films, about 8 μm thick, were exposed to ultraviolet rays from a sterilisation lamp at a distance of 3 cm in air of relative humidity 80% at 20 °C. The electric resistivity (measured in ohm) is that of the irradiated film surface of 1 cm². In the electrical resistance measurements, two silver plates (1 × 1 cm) were pressed on to the irradiated surface, at 1 cm apart, with a constant force. Since the photolytic silver existed predominantly on the irradiated surface and a little in the interior of the polymer film, the resistances between the irradiated surface and the rear surface, and of the rear surface scarcely changed with irradiation.

Thus, the amorphous films of silver salts are prepared on the glass plate, and, if necessary, can be easily peeled from the plate.

The films of all silver salts are colourless and transparent, and show a broad absorption band between 200 nm and 300 nm, which is associated with the charge transfer from carboxylate ions to silver ions. On irradiating with ultraviolet rays (200–300 nm), the films, at first, became brown coloured ($\lambda_{\text{max}} = 425\text{--}460\text{ nm}$) due to the formation of photolytic colloidal silver particles in the films. When irradiation was continued, however, the irradiated surfaces of the films gradually assumed metallic lustre and finally changed into clean silver mirrors. Because of the formation of silver mirrors, the surfaces of the sufficiently irradiated films had very high electric conductivities, which were about 10⁹ times as high as those of the original films.

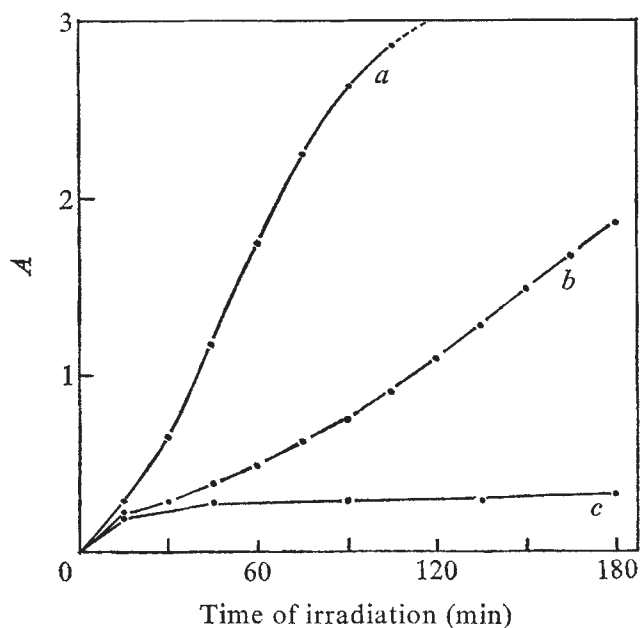


Fig. 2 Effect of water on the separation of silver for carboxymethylcellulose. The films were exposed in various humidities at 20 °C. The absorbance (A) was measured with a photographic densitometer. Chemical analysis confirmed that the optical density was proportional to the amount of the photolytic silver in the film. Humidities were, line a, 80%; b, 59%; c, 0%.

Figure 1 shows a plot of log resistivity of the irradiated surface of 1 cm² against time of irradiation for silver polyacrylate and silver carboxymethylcellulose. Similar results were obtained for the other silver salts in suitable conditions of irradiation. Since it is clear that the photolytic product which gives high electric conductivity on the surface is metallic silver, it is suggested that silver atoms produced by irradiation aggregate to form the colloidal silver particles near the irradiated surface and finally form a thin layer of metallic silver on the surface^{6,7}.

Water has a very important role in these photolyses. When irradiation was carried out in a dry box, the separation of silver occurred very poorly even on the prolonged irradiation (Fig. 2), and hence the surface of the film did not exhibit the metallic lustre. To form a silver mirror and increase the electric conductivity on the surface, it is necessary to irradiate the film under an atmosphere of the high relative humidity. It is clear that the water content in the film is closely related to the relative humidity in which it is placed. It seems likely, therefore, that the effect of water results from the action of water as a plasticiser, which facilitates

the segmental motion of the polymer and restrains the recombination of the primary products, that is, silver atoms and acyloxyl radicals.

YOSHIAKI KONISHI
HIROSHI SAJO

Department of Chemical Engineering,
Tokushima University, Tokushima

HIROSHI HADA
MIKIO TAMURA

Department of Industrial Chemistry,
Kyoto University, Kyoto, Japan

Received 5 May; accepted 5 July 1977.

¹ Finch, A., Jacobs, P. W. M. & Tompkins, F. C. *J. chem. Soc.* 2053–2060 (1954).

² Kubal, J. *Nature* 193, 1175 (1962).

³ Konishi, Y. & Saijo, H. *Chem. Lett.* 137–140 (1974).

⁴ Konishi, Y., Hada, H. & Tamura, M. *J. chem. Soc. Japan* 86, 1132–1135 (1965); 90, 992–996 (1969); 92, 829–833 (1971); 92, 1091–1095 (1971).

⁵ Hada, H., Ikenoue, S. & Tamura, M. *J. chem. Soc. Japan* 72, 145–147 (1969).

⁶ Tamura, M., Hada, H. & Konishi, Y. *J. chem. Soc. Japan* 86, 1135–1137 (1965).

⁷ Hada, H., Konishi, Y. & Sato, I. *Electrography (Denshi Shashin)* 13, 30–33 (1974).

Particulate transport through the mesosphere and stratosphere

THE 'aerosol layers' frequently observed at about 50 km altitude may be due to downward transport from above of particulates, possibly of extraterrestrial origin. This transport would be more efficient at higher altitudes (the mesosphere) than lower altitudes (the stratosphere) thus causing the layering seen in the stratopause region. The flux of particulates through the stratosphere provides a potential scavenging mechanism for pollutants and a source of condensation nuclei to the troposphere. Electric field variations, probably induced from above, can modulate this flux, providing a possible coupling mechanism between geomagnetic activity and terrestrial weather. The particulate distribution is also affected by the general circulation and atmospheric dynamics. The density of particulates is much greater than generally recognised, indicating that their role in chemical and thermal processes should be reassessed.

Noctilucent clouds provide visible evidence of the occasional presence of particulate matter in the high latitude summer mesopause region, but there is also evidence for the continual presence at many latitudes of a high density of very small (<10-nm) aerosol particles in the mesosphere and stratosphere. This evidence includes unexpectedly low and variable ion mobility and conductivity observations (refs 1–6) and ionospheric data such as the normally low density of electrons in the mesopause region⁴ and the very rapid loss of electrons during solar eclipse totality⁷. Features of the 'winter anomaly' in ionospheric radio wave absorption, such as the large variability⁴ and solar zenith angle dependence⁴ of electron density, can be explained by the absence of these particulates on 'anomalous' days.

Optical measurements, using a variety of techniques (Lidar, rocket and satellite scattering measurements at various wavelengths), have indicated 'aerosol layers', frequently in the vicinity of 50 km (ref. 8 contains references to earlier observations). This layering in the stratopause region may be explained by the more effective downwards transport in the mesosphere than the stratosphere of particulate matter, which may be of cosmic origin, possibly coated with ice or water⁴. Gravitational settling of 10-nm particles is less than 10^{-1} m s^{-1} at 80 km, decreasing rapidly at lower altitudes⁹. The dominant diffusion process in the stratosphere and mesosphere is 'eddy diffusion' and the applicable diffusion coefficient is much higher in the mesosphere than the stratosphere, which could produce layering

due to 'piling up' in the transition region, the stratopause. The expected eddy diffusion coefficient for the mesosphere¹⁰ yields characteristic downward velocities of order 10^{-2} m s^{-1} , implying a trans-mesospheric transit time of order 30 d. A more rapid process might involve the vertical electric field, which is generally expected to be downward in the upper atmosphere¹¹. To provide a net downward transport there must be an excess of positively- over negatively-charged aerosol particles (or a differential mobility). An excess of positively-charged particles would be expected since free electrons form in this region due to detachment (or emission) from negative ions. We need not rely on speculation on this point, however, since Widdel *et al.*², using a parachute-borne Gerdien condenser, observed positive ions in the mesosphere with mobilities an order of magnitude less than that of the small positive ions, but did not observe the corresponding low mobility negative ions, indicating that negative particulates did not form, or, more likely, were of very short lifetime due to electron detachment. A consensus of the properties of the particulates in the mesosphere⁴ would indicate a density of 10^9 – 10^{10} m^{-3} of less than 10 nm diameter and a mass of several thousand AMU. Recent measurements of the vertical electric field indicate a field of the order 1 V m^{-1} in the stratosphere, with some intensification in the mesosphere¹². Assuming a 1-Vm^{-1} field and mobilities for the particulate ions as for the lower mobility group seen by Widdel *et al.*², of $1 \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$ at 70 km (10^{-1} at 60) yields velocities of the order of 1 m s^{-1} at 70 km (10^{-1} at 60). Since at least 10% of the available aerosol particles can be expected to be charged (deducible from refs 2, 4, 13), this will provide a transport mechanism at least comparable with eddy diffusion if the electric field measurements are correct.

Recent measurements by Hirono *et al.*¹⁴, using both Lidar and direct electrical measurements on balloon-sondes, indicate a population of 'Aitken' particles in the stratosphere having similar densities ($\sim 5 \times 10^9 \text{ m}^{-3}$) to those deduced above for the mesosphere, with a relatively constant density (to a factor of 2) from the tropopause up to 30 km. The eddy diffusion coefficient is much smaller in the stratosphere¹⁵ and the trans-stratospheric transport by this mechanism would be expected to take years. The density of particulates would imply a downward flux of the order of $10^6 \text{ m}^{-2} \text{ s}^{-1}$. The corresponding electric field transport mechanism is hindered in this region by much lower mobilities (inversely proportional to air density), and is dependent for net transport on a differential density or mobility effect between positively and negatively charged particulates, as before. It would be expected that there might be some differential density effect due to detachment of electrons, as in the mesosphere. Assuming a reduced (STP) mobility of $3 \times 10^{-3} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$ (ref. 2), the downward velocity would be $3 \times 10^{-2} \text{ m s}^{-1}$ at 18 km for a 1 V m^{-1} electrical field, and would be constant above this for the usual assumption that the electric field decreases exponentially with altitude in the stratosphere, consistent with the observation of a relatively uniform particulate density¹⁴. For a 10% net differential density of positive over negative particulates the downward flux would be of the order of $1.5 \times 10^7 \text{ m}^{-2} \text{ s}^{-1}$ and trans-stratospheric transit would take about 10^2 d . Disturbances of the field, such as have been observed in connection with geomagnetic activity (ref. 16 and R. Mühleisen, personal communication) could modulate or interrupt the flow of condensation nuclei into the troposphere. This is the sort of mechanism which has been suggested to explain possible connections between solar and geomagnetic activity and weather^{17,18}.

The distribution of particulates would of course also be modified by atmospheric dynamics and the general circulation. One scenario for the 'winter anomaly' in radio wave absorption in the mesosphere would involve the depletion of particulates in sub-auroral regions by an enhanced or

reversed vertical electric field, with these regions then being transported by high-velocity equatorward winds. The early winter downdraft at mid-latitudes¹⁹ would be expected to move particulates into the troposphere. A wave-like structure in conductivity observed at a low-latitude site³ can be explained by a wave-induced bunching of particulates.

The presence of such a high density of particulates in generally downward flow throughout the mesosphere and stratosphere shows that their role in chemical and thermal processes should be evaluated, but this remains uncertain without knowledge of their structure and composition. For example, their effectiveness in scavenging could be greatly enhanced if they are water coated.

It may be that the 'fair weather' electric field expected at the tropopause could be perturbed using high d.c. voltages on high electrodes or antennae, possibly using tethered balloons. Less than 10^6 V on an electrode 1 km above its image would produce an electric field sufficient to double or cancel the expected tropopause electric field ($1\text{--}10\text{ V m}^{-1}$) at any latitude. This suggests a means of testing for an interaction between the vertical electric field and atmospheric processes with Lidar or sonde measurements.

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LESLIE C. HALE

*Ionosphere Research Laboratory,
The Pennsylvania State University,
University Park, Pennsylvania 16802*

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- ¹ Bourdeau, R. E., Whipple, E. C., Jr & Clark, J. F. *J. geophys. Res.* **61**, 1363 (1959)
- ² Widdel, H. U., Rose, G. & Borschers, R. *Pure appl. Geophys.* **84**, 154 (1971).
- ³ Bragin, Yu. A. *Nature* **245**, 450 (1973).
- ⁴ Chesworth, E. T. & Hale, L. C. *Geophys. Res. Lett.* **1**, 347 (1974).
- ⁵ Croskey, C. L., Hale, L. C. & Leiden, S. C. *Space Res.* **17**, (in the press).
- ⁶ Mitchell, J. D., Sagar, R. S. & Olsen, R. O. *Space Res.* **17**, (in the press).
- ⁷ Narcisi, R. S., Bailey, A. D., Wlodyka, L. E. & Philbrick, C. R. *J. atmos. terr. Phys.* **34**, 647 (1972).
- ⁸ Giovane, F., Schuerman, D. W. & Greenberg, J. M. *J. geophys. Res.* **81**, 5383 (1976).
- ⁹ Kellogg, W. W. *Space Sci. Rev.* **3**, 275 (1964).
- ¹⁰ George, J. D., Zimmerman, S. P. & Keneshea, T. J. *Space Res.* **12**, 695 (1972).
- ¹¹ Dolezak, H. *Pure appl. Geophys.* **84**, 9 (1971).
- ¹² Tyutin, A. A. *Cosmic Res.* **14**, 132 (1976).
- ¹³ Castleman, A. W., Jr *Space Sci. Res.* **15**, 547 (1974).
- ¹⁴ Hirono, M., Fujiwara, M. & Itabe, T. *J. geophys. Res.* **81**, 1593 (1976).
- ¹⁵ Hunt, D. M. *Geophys. Res. Lett.* **2**, 26 (1975).
- ¹⁶ Reiter, R. J. *atmos. terr. Phys.* **39**, 95 (1977).
- ¹⁷ Roberts, W. O. & Olson, R. H. *J. Atmos. Sci.* **30**, 135 (1973).
- ¹⁸ Dickinson, R. E. *Bull. Am. met. Soc.* **56**, 1240 (1975).
- ¹⁹ Houghton, J. T. *Presidential Address, Royal met. Soc.* (1977).

Novel method for direct analysis of hydrocarbons in crime investigation and air pollution studies

MODERN methods for sampling of organic vapours in air pollution studies avoid the problems caused by the condensation of water vapour, by collection at ambient temperature on charcoal¹, porous polymers²⁻⁵ or support-bonded phases³. The tenacity of adsorption renders the sample difficult to remove and indirect methods such as liquid extraction^{1,5} or thermal desorption²⁻⁴ are necessary to produce a sample for gas chromatographic (GC) or GC-mass spectrometric analysis. We describe here an analytical technique based on the Curie point pyrolysis system⁶ which overcomes these problems by using ferromagnetic wires coated with a thin layer of highly active charcoal. These are simply suspended in the atmosphere under test for 1-2 h at room temperature, placed in the Curie point apparatus and then inductively heated in the normal manner. Thus the sample is rapidly introduced into the GC for direct analysis. The fact that the total amount of each hydrocarbon present is not adsorbed by this method is outweighed by the advantages of direct sample intro-

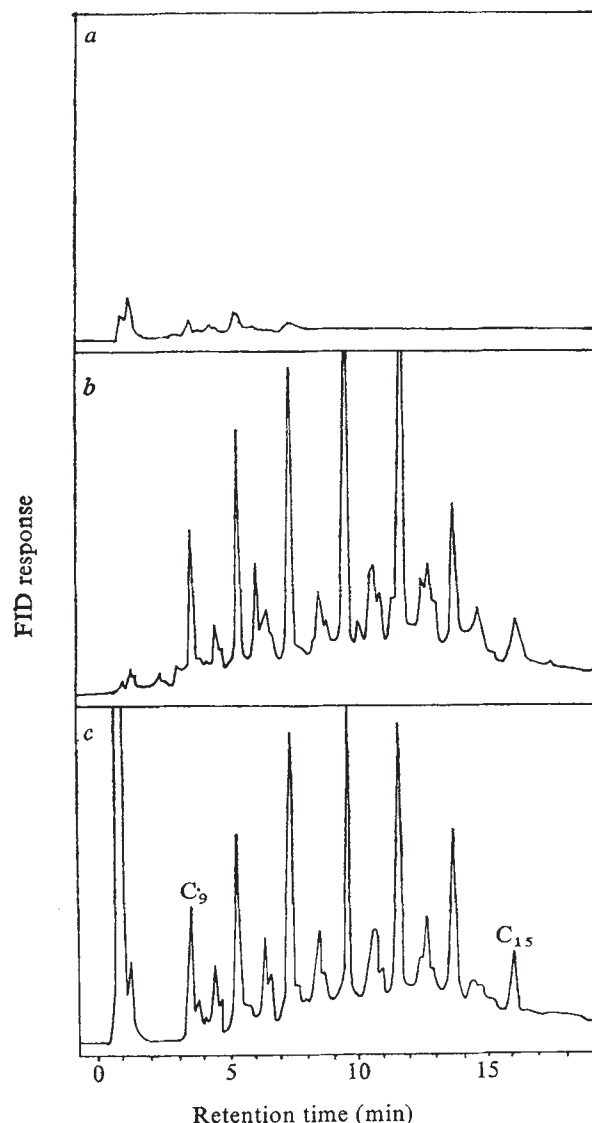


Fig. 1 Gas chromatograms obtained from *a*, 1-ml aliquot taken with heated syringe from a 180-ml glass bottle sealed with nylon film, containing 5 μ l paraffin and heated to 110 °C; *b*, induction heating for 10 s in the Curie point apparatus of an adsorption wire after 1.5 h immersion in an identical headspace to (*a*), at 22 °C, the chromatogram being obtained at the same attenuator settings as above; *c*, solution injection of the same paraffin sample for reference. Gas chromatography was carried out on a Pye 104 instrument fitted with flame ionisation detectors (FIDs) using 10-foot $\times$ $\frac{1}{8}$ -inch columns of 15% carbowax 20M on Chromosorb W. A flow rate of 60 ml per min of nitrogen was maintained and the column programmed from 70–180 °C at 8 °C per min. Adsorption wires were prepared by bonding finely divided (passed 240 mesh) highly active carbon (Norit SX1, Hopkin and Williams) to nickel (358 °C Curie point) wires with a high temperature inert adhesive (Cement Binder LQ/S6, Fortafix Ltd). The dried wires were conditioned by several 10-s inductive heating periods in a Pye Curie point apparatus connected directly to the GC column in the normal manner. Wires are ready to use when no further peaks are observed on the GC trace with a subsequent induction heating period. After removal from the apparatus and exposure of an adsorption wire to the vapours under examination, it is returned to the apparatus. The wire is inductively heated for 10 s and the column temperature programming started. At the end of the GC run the wire can be reconditioned for reuse by further repeat inductive heating periods as above. Although 358 °C Curie point wires were chosen initially to avoid pyrolysing the adsorbed material, limited use of 610 and 770 °C Curie point wires suggests that this is not a problem with the hydrocarbon mixtures examined. It is advisable to store conditioned wires over freshly-activated charcoal.

duction, which offers distinct advantages to the forensic scientist in the investigation of a suspected arson. The technique could also find quantitative application in air pollution studies.

In forensic examination of fire debris for accelerant residues, quantitation has little relevance, the main objectives being to identify the type of accelerant, and where required compare it with a control sample. Current methods rely on the relatively high vapour pressure of hydrocarbons to separate them from the debris. Headspace analysis⁷, by direct injection of vapour aliquots taken from a heated nylon bag containing the debris, can often indicate the type of accelerant used. Limitations are imposed on this method by the decrease in vapour pressure of the hydrocarbon species with increasing molecular weight. Thus the less volatile accelerants give poor headspace chromatograms and depletion of the more volatile constituents during a fire creates further difficulties in the identification of the original accelerant. Other techniques involve thermal desorption of the residues followed by cold trapping and are lengthy procedures; the condensate still requiring further processing before analysis. Adsorption traps (J. B. F. Lloyd, personal communication), while improving the procedure again do not provide direct sample introduction. The carbon adsorption wire technique is eminently suited to arson investigation, as it retains the simplicity of headspace analysis whilst producing a much more representative chromatogram, especially for the less volatile material present in the sample.

Figure 1 shows comparisons obtained from identically prepared paraffin vapour samples using hot headspace analysis (Fig. 1a) and using an adsorption wire at room temperature (Fig. 1b). These comparisons are made at the same instrument attenuation in order to demonstrate the concentrative effect of the wire. The results show that to obtain a response for *n*-decane equivalent to that obtained by the wire 21 ml of hot headspace would be required. Less volatile species show further improvement; for

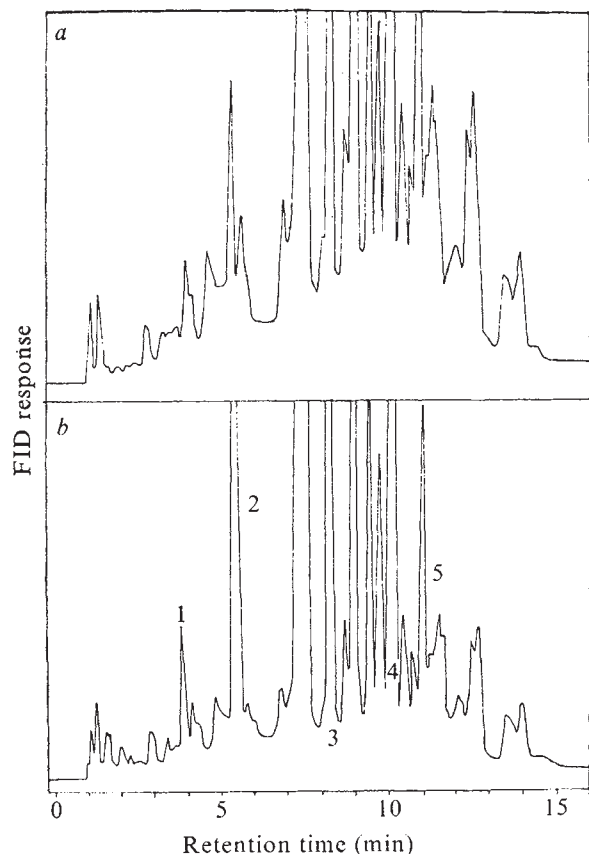


Fig. 2 Adsorption wire chromatograms obtained (as described in Fig. 1) from *a*, petrol sample from which 90% of the material had previously been allowed to evaporate in an open dish; *b*, from the original petrol sample. (Numbered peaks are referred to in Fig. 3.)

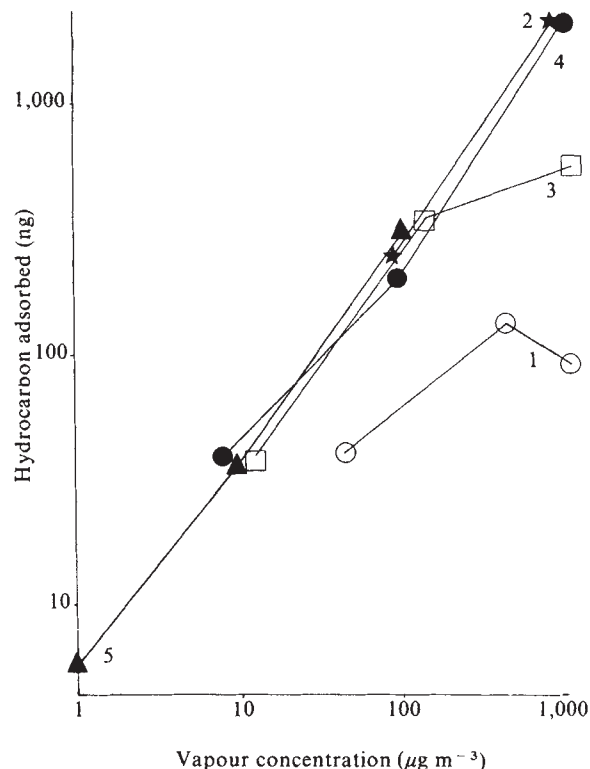


Fig. 3 Amounts collected by an adsorption wire versus vapour phase concentration for various species (log scales) after insertion for 1 h in a 100 l nylon bag containing known dilutions of petrol vapour in air. 1, Benzene; 2, toluene; 3, *o*-xylene; 4 and 5, trimethylbenzenes. Other aromatic species examined (not shown for clarity) fit the same calibration line as 2-5.

n-hendecane and *n*-dodecane, 55 and 200 ml respectively of headspace would be required. Comparison of the wire chromatogram with that of the liquid sample (Fig. 1c) demonstrates the wire's ability to adsorb the entire range of components present in a paraffin sample producing a chromatogram closely resembling the original material. Although the adsorption wire chromatogram shows slight differences in fine structure from the liquid paraffin sample, extremely accurate reproduction occurs in the case of petrol samples at the less volatile end. This may be due to slight preferential adsorption of the aromatic species by the carbon, and presents no problems for paraffin samples providing any samples for comparison are also examined by the adsorption wire method.

The ready adsorption of less volatile materials by the carbon overcomes the problems encountered by headspace analysis in dealing with residues which have been extensively evaporated in a fire. Figure 2 compares adsorption wire chromatograms obtained from a 90% evaporated petrol sample (Fig. 2a) with that from the original petrol (Fig. 2b). The only differences observed are at the more volatile end, the remainder of the chromatograms showing identical fine structure. This demonstrates the considerable advantages of the technique for the recognition of heavily evaporated accelerants in crime investigation.

Rapid adsorption of vapour phase components occurs with these wires, characteristic petrol traces being obtainable after only a few seconds immersion in a strong vapour. For the less volatile components of a paraffin headspace immersion for about 1.5 h is necessary to obtain a representative chromatogram, as the situation is complicated by adsorption on the vessel walls or in the debris. Where the vapours could not escape no further change has been observed in the wire chromatogram on longer periods of immersion.

The technique has been used successfully to identify

petrol and paraffin residues in simulated fires with pine sawdust, when headspace analysis failed to produce recognisable traces. It has also been used as a rapid means of introducing retention index standards for calibrating Curie point pyrolysis GC. For this application the wires are held in the vapour space above each liquid sample for several seconds, separately for individual species, or sequentially for a series of materials. In this way the samples may be introduced in identical manner to that for the pyrolysis products, and changing of the inlet system is not required.

The technique has obvious application to air pollution analysis as a means of collecting and identifying components in a stack gas or other emission source. The method can be calibrated and has sufficient sensitivity for the quantitative monitoring of ambient air. Repeat experiments with the same wire immersed in equivalent volumes of known concentrations of petrol vapour in air gave the results displayed graphically in Fig. 3. Although the relationship between the amount adsorbed and the gas phase concentration was nonlinear, the adsorption characteristics of aromatic species studied were remarkably similar, with the exception of benzene. At levels exceeding $100 \mu\text{g m}^{-3}$ (about 500 ng adsorbed on the treated wire) saturation effects began to appear for toluene and the xylenes, but below this level a calibration based on a mean line for all species (except benzene) would apparently result in errors of less than a factor of two. These results suggest that the technique may be used down to levels of $1 \mu\text{g m}^{-3}$ which for the range of species examined is equivalent to 0.2–0.3 p.p.b. (volume parts per 10^9).

Depending on the species and site locations, city levels of these species lie in the range 0.3–130 p.p.b. (refs 1, 4, 8). Thus the method has the required sensitivity for ambient air monitoring although further work would be necessary to establish the performance of these adsorption wires in the dynamic environment of a city street.

J. D. TWIBELL
JANET M. HOME

Home Office Central Research Establishment,
Aldermaston, Reading, Berkshire, UK

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¹ Grob, K. & Grob, G. J. *Chromat.* 62, 1–13 (1971).

² Dravnieks, A., Krotoszynski, B. K., Whitfield, J., O'Donnell, A. & Burgwald, T. *Environ. Sci. Technol.* 5, 1220–1222 (1971).

³ Perry, R. & Twibell, J. D. *Atmos. Env.* 7, 929–937 (1973).

⁴ Bertsch, W., Chang, R. C. & Zlatkis, A. J. *Chromatog. Sci.* 12, 175–182 (1974).

⁵ Aue, W. A. & Teli, P. M. J. *Chromat.* 62, 15–27 (1971).

⁶ Simon, W. & Giacobbo, H. *Angew. Chem. Intern. Ed.* 4, 938–943 (1965).

⁷ Ettling, B. V. & Adams, M. F. J. *Forensic Sci.* 13, 76–89 (1968).

⁸ Lonneman, W. A., Bellar, T. A. & Altschuller, A. P. *Environ. Sci. Technol.* 2, 1019–1020 (1968).

The Asian source of Arctic haze bands

'ARCTIC haze' refers to turbid layers of air which are found regularly over the pack ice north of Alaska during periods of clear weather¹. These layers are diffuse, hundreds to thousands of kilometres wide, 1–3 km thick, and can occur as single or multiple bands of different heights at nearly any level in the troposphere. They are invisible from the ground, but may limit horizontal and slant visibility within a layer to as little as 3–8 km. Their colour is grey-blue in the antisolar direction and reddish-brown in the solar direction, suggesting that they are true aerosol rather than ice crystals.

The initial, purely visual observation of Arctic haze were made more than 20 years ago. It was then forgotten about until 1972 when radiation measurements near Barrow, Alaska revealed unexpectedly high atmospheric turbidities, confirmed in 1974 (ref. 2). The anomalous turbidity was partly found in distinct layers at altitudes of only a few

kilometres. About 40%, however, was above 4 km, the effective ceiling of the aircraft used.

The 1974 observations raised the question of possible anthropogenic origin of the haze, because rough trajectory analysis for a persistent haze period during March and April 1974 suggested that the air had passed over the north-eastern United States some days earlier. To check this possibility, we have used the chemical composition and morphology of the spring 1976 Arctic aerosol as indicators of its source, and concluded that it originated in the great Asian deserts. During April and May 1976 a series of 15 flights with a single-engine aircraft were made from the Naval Arctic Research Laboratory in Barrow, Alaska. Nine high-volume (90–360 m³) samples of haze aerosol were collected on acid-washed 11-cm Whatman No. 41 cellulose filters. Nuclepore filter samples were taken concurrently for electron microscopy. Height of the haze layers was determined by a combination of visual observation, condensation-nucleus count, and turbidity profile.

The high-volume samples were subsequently analysed at the University of Rhode Island for a number of elements with short-lived nuclides by neutron activation, with results shown in Table 1. The month-long sampling period had very low elemental concentrations at the beginning, a strong maximum in the middle, followed by a sharp dropoff at the end. The high-concentration period coincided with visible haze bands, all of whose visual properties suggested Arctic haze. The bands did not come and go quickly; rather a single broad maximum of 6–13 days' duration was seen. During this period Al and Mn were increased in concentration relative to their initial and final values by over an order of magnitude, V by an order of magnitude, and Na and Ba by somewhat less than an order of magnitude.

The aerosol-crust enrichment factors (Table 1) for the elements

$$Ef_X(\text{Al, rock}) = (X/\text{Al})_{\text{aerosol}} / (X/\text{Al})_{\text{rock}}$$

showed similar trends of high values in the early samples which decreased smoothly to unity or lower with increasing haze aerosol. For V the trend was most marked; indeed of these elements V has the highest enrichments in cities (5–500 (ref. 3)). Taken together, the trends of these enrichment factors clearly indicated that the background aerosol was pollution-derived but that the haze aerosol itself was crustal, that is natural, just the opposite of what we had expected. Electron microscopy of the Nuclepore filters showed that angular crustal particles of diameter greater than roughly 0.4–0.8 μm are present in all samples, but with greatly increased numbers during the haze episode. In contrast, most particles smaller than 0.4–0.8 μm diameter were nearly spherical; their abundances were much less haze-dependent. Although we have studied only this one episode, we feel that it may well be typical, as discussed below.

The source of the crustal haze aerosol must be very strong to account for its high concentrations. It seems to be the great Takla Makan and Gobi deserts of eastern Asia. Upper-level constant-pressure charts indicate that our haze period was preceded by several days of intense air flow from these deserts to Alaska. Figure 1 shows the 700-mb isobaric trajectories for air arriving at Barrow before, during, and after the episode. Only episodic air had recently passed over the Asian deserts.

Several factors support the idea of an Asian source for Arctic haze. First, spring is the period of greatest dust storms in deserts. In our case, during nearly all of April 1976 the Asian deserts were filled with dust storms. Second, the composition of Arctic haze greatly resembles that of the Sahara aerosol (K. A. Rahn, L. Schütz, and R. Jaenicke, unpublished data). Third, flow patterns conducive to long-range transport from Asia to Alaska are strongest

Table 1 Elemental concentrations and enrichment factors for aircraft filter samples

Sample	AB	CD	E	F	G	IJ	KL	M	N
	Concentration [ng m^{-3} (ambient)]								
Al	10.0 $\pm$ 0.9	17.0 $\pm$ 1.0	35 $\pm$ 2	34 $\pm$ 2	91 $\pm$ 5	240 $\pm$ 10	203 $\pm$ 10	240 $\pm$ 10	14.7 $\pm$ 1.7
V	0.035 $\pm$ 0.006	0.148 $\pm$ 0.010	0.126 $\pm$ 0.012	0.094 $\pm$ 0.010	0.22 $\pm$ 0.02	0.40 $\pm$ 0.03	0.37 $\pm$ 0.02	0.40 $\pm$ 0.04	0.0192 $\pm$ 0.0100
Mn	0.146 $\pm$ 0.012	0.36 $\pm$ 0.02	0.59 $\pm$ 0.04	1.58 $\pm$ 0.03	1.55 $\pm$ 0.08	3.0 $\pm$ 0.2	3.1 $\pm$ 0.2	3.4 $\pm$ 0.2	0.21 $\pm$ 0.02
Na	12 $\pm$ 10	32 $\pm$ 11	<30	21 $\pm$ 16	47 $\pm$ 27	58 $\pm$ 15	37 $\pm$ 11	37 $\pm$ 30	<20
Ba	0.4 $\pm$ 0.2	0.4 $\pm$ 0.2	<0.8	<0.5	1.6 $\pm$ 0.6	2.0 $\pm$ 0.2	2.0 $\pm$ 0.2	1.9 $\pm$ 0.5	<0.5
	Enrichment factor (Al, rock)								
Al	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
V	2.1	5.2	2.2	1.66	1.46	1.00	1.10	1.00	0.79
Mn	1.25	1.81	1.44	1.46	1.46	1.07	1.31	1.21	1.22
Na	3.4	5.4	<2.5	1.77	1.48	0.69	0.52	0.44	<4
Ba	8	5	<4	<3	3.5	1.7	1.8	1.5	<6
Date	12, 13 April	14, 15 April	17 April	19 April	21 April	28, 29 April	30 April, 1 May	3 May	5 May
Volume (m^3)	249	341	149	176	110	309	365	88.7	108
Altitude (km)	3.3	2.0, 1.2	2.1	2.1	3.0	1.8, 2.3	2.1, 2.0	2.5 to 2.8	1.8

during the spring^{4,5}. Fourth, the Asian deserts are farther north than the other deserts, lying mostly between 40° and 50° North as opposed to the more normal desert latitude of 20° to 30°. Fifth, the length of typical trajectories between Asia and Alaska (9,000–12,000 km) is not out of line with the well documented 6,000-km path for transport of Sahara dust to Barbados⁶.

An Asian source for Arctic haze explains or confirms many previous observations such as:

(1) Anomalously high turbidities over Barrow, especially during the spring⁷ (flow patterns between Asia and Alaska appear to be common from November through May);

(2) The large size of the haze bands (see below);

(3) Previous haze events at Barrow (for example, the episode of March–April 1974 was preceded by a few days of strong flow from the Asian deserts);

(4) Previous measurements of anomalous turbidities over Fairbanks, Alaska. For example, 17–22 February 1976 was a whitish haze episode in Fairbanks which affected air at all levels and for which no explanation could be found.

Visibilities were reduced from the normal 150 km to less than 30 km. Trajectory analysis now reveals that this air was also of Asian origin. Similar haze incidents with trajectories leading back to Asia seem to be a regular feature of the Fairbanks atmosphere during winter and spring;

(5) The accumulation of brownish insoluble deposits in the pack ice north of Barrow, which under examination with a light microscope seem to be continental dust (R. Paquette, personal communication). The mineralogy and possible sources of this dust have also been discussed⁸;

(6) Anomalous ice-nucleus concentrations at College, Alaska, Blue Glacier, Washington, and Nagoya, Japan during February and March 1968, for which trajectory analysis showed eastern Asia to be the probable source⁹;

(7) A case of abnormally high condensation nucleus concentrations at Barrow in March 1970¹⁰, which was explained as pollution from Prudhoe Bay to the east but which may have originated from Asia because the large-scale flow for this period was from those deserts.

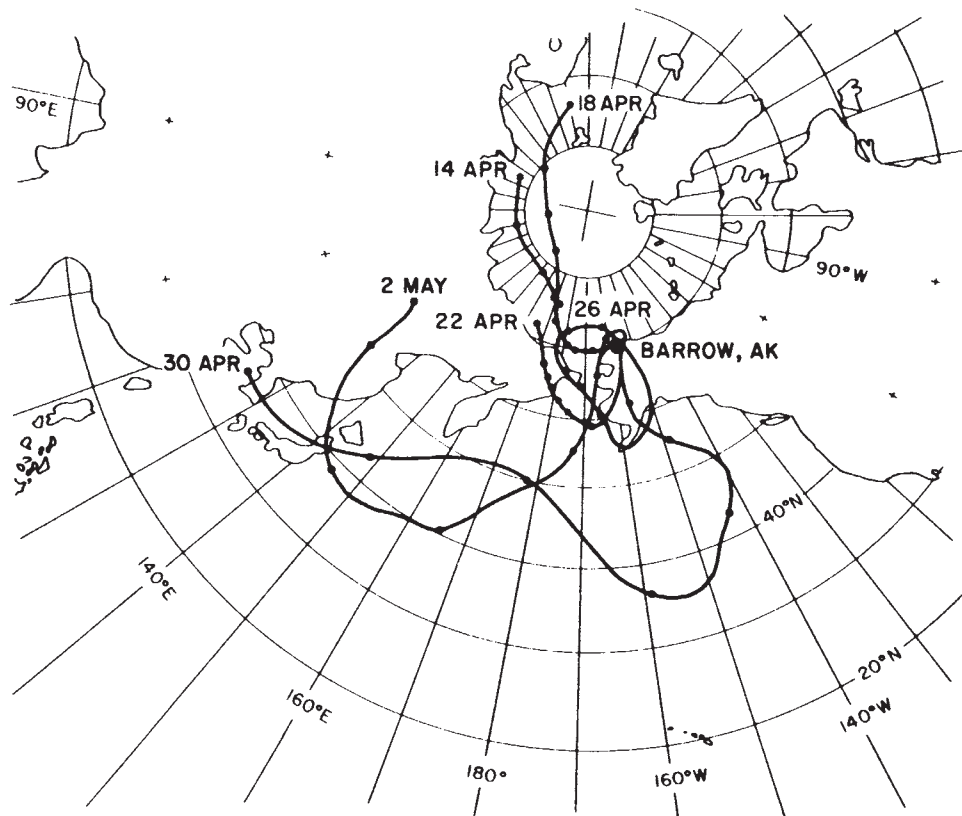


Fig. 1 700-mbar isobaric trajectories of air to Barrow. Numbers refer to date of arrival at Barrow. Solid circles each 24 hours along trajectory.

The mass of desert dust transported into the Arctic seems to be very great. For the five-day episode over Fairbanks discussed above, columnar mass loadings derived from radiation measurements showed values of about 45 mg m^{-2} (for aerosol density 2.5). For a point source in Asia and a dispersion angle of 5° (typical of volcanic plumes), the dust cloud would be 900 km wide at Alaska, or about the width of the state itself. (Indeed during this incident visibility was poor over the entire north half of the state.) Such a plume travelling at 80 km h^{-1} would carry 4,000 tons of dust into the Arctic per hour, or a half-million tons over the five-day episode. This is equivalent to skimming off a $0.2\text{-}\mu\text{m}$ layer from a $10^\circ \times 10^\circ$ desert.

Clearly, more detailed studies are needed to refine our ideas about this significant phenomenon. Also, possible climatic effects of such large amounts of aerosol in the Arctic should not be overlooked.

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KENNETH A. RAHN
RANDOLPH D. BORYS*

Graduate School of Oceanography,
University of Rhode Island,
Kingston, Rhode Island, 02881

GLENN E. SHAW

Geophysical Institute,
University of Alaska,
Fairbanks, Alaska, 99701

Received 17 January; accepted 22 April 1977.

*Present address: Department of Atmospheric Sciences, Colorado State University, Fort Collins, Colorado 80522.

- 1 Mitchell, M. J. *Atmos. Terr. Phys. Special Supplement* 195–211 (1956).
- 2 Holmgren, B., Shaw, G. & Weller, G. *AIDJEX Bulletin* 27, 135–148 (1974).
- 3 Rahn, K. A. *The chemical composition of the atmospheric aerosol* (University of Rhode Island Technical Report, 1976).
- 4 Staff members of the section of Synoptic and Dynamic Meteorology, Institute of Geophysics and Meteorology, Academia Sinica, Peking, *Tellus* 9, 432–446 (1957); *ibid.* 10, 58–75 (1958).
- 5 Jackson, M. L. *et al. Soil Sci.* 116, 135–145 (1973).
- 6 Carlson, T. N. & Prospero, J. M. *J. appl. Meteor.* 11, 283–297 (1972).
- 7 Shaw, G. E. *Tellus* 27, 39–49 (1975).
- 8 Darby, D. A., Burckle, L. H. & Clark, D. L. *Earth planet. Sci. Lett.* 24, 166–172 (1974).
- 9 Isono, K. *et al. Tellus*, 23, 40–59 (1971).
- 10 Radke, L. F., Hobbs, P. V. & Pinnons, J. E. *J. appl. Meteor.* 15, 982–995 (1976).

Sulphur dioxide discharge from Mount Etna

REMOTE sensing of SO_2 emitted by Mount Etna in its normal state of permanent activity, indicates much higher flow-rates than those previously measured by the same method (correlation spectrometry) on other volcanoes. A set of measurements in June 1975 provided a mean SO_2 flow-rate of $3,740 \text{ t d}^{-1}$ for this volcano. This is a striking result both for volcanology and for environmental sciences. The knowledge of the mass discharge of chemical species from an active volcano and of its variations, both in time and space, is of great interest for the understanding of eruptive mechanisms and the establishment of geochemical balances. The SO_2 content of a volcanic plume can be measured from a distance by an optical correlation technique; this method was perfected a few years ago and has since been widely used for the remote sensing of air pollution in industrial areas¹. During June 1975, we used the correlation spectrometer Barringer Cospec II to measure the SO_2 emitted by the active vents in the summit of Mount Etna (Central Crater, Bocca Nuova, North-East Crater and 1964 Crater), 3,200 m above sea level.

Activity within these craters was then characterised by a strong emission of condensed water vapour. From time to time, the plume issuing from the Central Crater was

loaded with ashes produced by explosive activity taking place at least 500 m beneath the crater rim. At 2,600 m above sea level, on the northern flank of the volcano, the lateral eruption which had begun in February 1975 was still in progress, characterised by a slow effusion of an almost entirely degassed lava.

The remote sensing correlation spectrometer compares a high contrast reference spectrum (multiple slit mask) with the molecular absorption spectrum of the gas mixture examined (in the $3,100\text{\AA}$ region for the sensing of SO_2). This correlation technique ensures the sensitivity and the selectivity of the instrument over a broad range of concentrations. The Cospec II displays the concentration times path length product of the SO_2 present along its optical path. In other words, the response of the spectrometer is directly proportional to the product p.p.m. (mean concentration of SO_2 in the sighted plume) $\times$ m (thickness of the plume) according to the Beer–Lambert's law. The skylight is the ultraviolet source for the spectrometer. Weak variations in the intensity of this source are automatically compensated; the stability of the instrument, in the absence of any cloud cover is maintained from three hours after sunrise until 3 h before sunset. Two quartz cells, containing the gas to be detected in two different concentrations, can be set on to the optical axis of the remote sensor: they are an internal calibration of the spectrometer. This internal calibration can be checked by sighting with the spectrometer just above the stack exit of a fossil fuelled power plant, whose SO_2 emission is accurately known (true scale external calibration).

On Mount Etna, measurements were made by the traverse method whereby the remote sensor equipped with its vertical sighting device is carried by car under the plume. A continuous westerly wind throughout our series of measurements provided the best meteorological conditions available on this site: the plume was drifting over a nearly straight motorway at sea level, 10 km downwind from the volcanic discharge area, on which the car could travel at constant speed between the cities of Giarre and Taormina. For each traverse, the signal of the spectrometer was recorded continuously. The SO_2 concentration of the plume appears on the strip chart as a nearly gaussian curve; its area is the product of concentration by surface (p.p.m. $\times$ m²). From this measurement—corrected by taking into account the mean angle between the motorway and the plume axis—and from the mean wind velocity, one can deduce for each traverse the mass flow of the detected gas. The wind velocity was measured by teleanemometry. The teleanemometer used has been designed and constructed at Bradford University. It consists of an optical telescope and two photosensitive cells which measure the shifting rate of an illumination difference between two points of the field of the telescope. The signals of the two cells are treated on a digital correlator which can extract a mean delay time corresponding to the velocity of the sighted plume.

Table 1 displays the measurements from Mount Etna made during three days. The following conclusions can be drawn. When a main road exists near the base of a volcano, the traverse method ensures little dispersion of the measurements. Therefore, a systematic use of the optical correlation technique for the remote sensing of SO_2 could be used to detect slight variations of volcanic gaseous discharge that might indicate changes in eruptive activity.

Table 2 compares the mean values of SO_2 discharge from Mount Etna (17 traverses in 3 d) with those obtained from other volcanoes using the same technique. The flow measured from Mount Etna seems to be much higher than that measured from Central American or Japanese volcanoes, despite apparently similar activity (that is, moderate pyroclastic activity) during the time of the measurements. Moreover, during violent pyroclastic activity, as emphasised

Table 1 SO₂ discharge from Mount Etna in June 1975

Date	Wind velocity (km h ⁻¹)	Traverse number	Time (beginning of traverse)	SO ₂ emission (t d ⁻¹)
14 June 1975	30 (estimated)	2	11.00	4800
		3	11.30	3800
		4	12.30	3200
		5	15.00	2700
		6	15.30	3900
		7	16.00	3600
		9	16.30	4200
		10	17.00	3700
15 June 1974	36 (measured)	12	11.40	3300
		13	12.00	4200
		14	12.45	3800
		15	13.00	4000
18 June 1975	31 (measured)	1	11.00	4100
		2	11.35	3700
		3	12.20	3600
		4	14.25	3600
		5	15.00	3400

The normal permanent activity provides a very stationary SO₂ flow within the time scale used (duration of traverse under the volcanic plume: approximately 10min).

by previous authors correlation spectrometer measurements cannot be made because ash-laden plumes screen the skylight. For these two reasons, it seems hazardous to assume a global SO₂ discharge rate to the atmosphere for all cases.

SO₂ seems to be the main sulphur species in a volcanic gas plume emitted at high temperature and without a great amount of ash or condensed water vapour which could act as an oxidation catalyst and lead to the conversion of SO₂ into sulphates. This SO₂ prevalence is indicated by the generally low value of the SO₄²⁻/SO₂ ratio obtained in volcanic gas plumes². Measurements by Le Guern³ on Mount Etna in June 1972 (activity similar to that of June 1975) showed that a sulphuric acid aerosol was emitted by the summit area of the volcano at a rate of 24 t d⁻¹. If as in fossil fuelled power plant fumes⁴, SO₂ in volcanic discharge remains at this oxidation state for a long time as compared with the delay time between emission and observation, one can assume that the SO₂ flow accurately represents the total sulphur discharge of an Etna-like volcano. Hence, in order to measure the flow of all other chemical constituents of the volcanic gas phase, it is sufficient to measure their concentration in the plume against SO₂ concentration.

Nevertheless, the question of the oxidation rate of SO₂ in volcanic gas plumes during atmospheric diffusion is still

unsettled. If this oxidation occurs more rapidly than mentioned above, then the flow rates reported in this paper are too low. In general, and essentially because of the light scattering by aerosols in the volcanic plume and in the atmosphere, all the SO₂ fluxes measured with the correlation spectrometer technique must be considered as minimums.

During our measurements of the SO₂ discharge from Mount Etna, the lava flow from the northern flank of the volcano had been estimated at 10⁴ t d⁻¹. Assuming that 0.07% sulphur by weight is emitted by a basaltic magma while degassing⁵, the estimated lava flow corresponds to a 14 t d⁻¹ SO₂ discharge from one of the summit craters of Mount Etna (in all likelihood the north-east one). This is far from the measured discharge and we may assume that the SO₂ originates from the degassing of a much greater mass of magma moving by convection within the volcano pipe. Using again the assumption of Moore and Fabbri⁶, the mass flow of this convecting magma would at least represent 2.7 × 10⁶ t d⁻¹ (~10⁶ m³). The more recent data¹¹ available on magmas more likely to reflect Etna's volatile content may suggest higher initial sulphur concentrations in the magma (up to 0.16% sulphur for basaltic liquids before effervescence). These data do not change the discrepancy between the observed lava effusion and the measured rate of outgassing.

Table 2 Comparison between SO₂ discharges from different volcanoes

Volcano	Date	SO ₂ emission (t d ⁻¹)	Ref.
O-Shima*	20 April 1971	345	6
Asama*	1 June 1972	142	7
Santiago†	1973	420	8
Fuego†	1973	40	8
Pacaya†	1973	260	8
San Cristobal‡	1973	360	8
Telica‡	1973	20	8
Momotombo‡	1973	50	8
Masaya‡	1973	180	8
Asama*	28 April 1974	787	9
Kilauea§	9-19 Feb. 1975	280	10
Sulfur Banks§	1975	7	10
Mauna Ulu§	1975	30	10
Etna	14, 15, 18 June 1975	3740	This paper

In all cases, the same sensing technique was used.

* Japan.

† Guatemala.

‡ Nicaragua.

§ Hawaii.

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R. HAULET
P. ZETTWOOG

Commissariat à l'Energie Atomique,
Département de Protection,
Service Technique d'Etudes de la Pollution
dans l'Atmosphère et dans les Mines,
B.P. No 6, 92260 Fontenay aux Roses,
France

J. C. SABROUX

Laboratoire de Volcanologie du CNRS,
Centre des Faibles Radioactivités,
B.P. No 1, 91190 Gif sur Yvette,
France

Received 11 May; accepted 28 June 1977.

- ¹ Moffat, A. J. & Millan, M. M. *Atmos. Environ.* **5**, 677-690 (1971).
- ² Caddle, R. D., Wartburg, A. F. & Grahek, F. E. *Geochim. cosmochim. Acta* **35**, 503-507 (1971).
- ³ Le Guern, F. *Comptes R. D.* **275**, 1867-1869 (1972).
- ⁴ Sabroux, J. C. thesis, Univ. Paris VI, 1974.
- ⁵ Moore, J. G. & Fabbri, B. P. *Contr. Mineral. Petrol.* **33**, 118-127 (1971).
- ⁶ Okita, T. *Japan Spectroscopic Co. Rep.* (Tokyo, 1971).
- ⁷ Moffat, A. J., Kakara, T., Akitomo, T. & Langan, L. *Air Note, Environ. Measurements Inc.* (San Francisco, 1972).
- ⁸ Stoiber, R. E. & Jepsen, A. *Science* **182**, 577-578 (1973).
- ⁹ Okita, T. & Shimozuru, D. *Bull. volcanol. Soc. Japan* **19**, 151-157 (1975).
- ¹⁰ Stoiber, R. E. & Malone, G. B. *Trans. Am. geophys. Union* **56**, 461 (1975).
- ¹¹ Anderson, A. T. *Geol. Soc. Am. Bull.* **85**, 1485-1492 (1974).

Superheavy element fission hypothesis in carbonaceous chondrites

IN many meteorites, the abundances of the heavy isotopes of Xe are enriched relative to solar Xe. In some cases, this enrichment results from the addition of fission products from U and Pu. In some carbonaceous chondrites, however, the isotopic enrichment pattern of the heavy Xe is inconsistent with the measured Xe yield patterns of U, Pu, Cm, and Cf fission. The lack of correlation between heavy Xe enrichment and U concentration in separated mineral phases also argues that the Xe is not due to fission of an element whose chemical properties are similar to U. It has been suggested that this enrichment resulted from the decay of a superheavy element^{1,2}. An experimental test of this hypothesis is proposed here.

Lewis *et al.* have determined that most of the trapped Xe in Allende and several other carbonaceous chondrites is contained in chromite, carbon and an unidentified phase, Q, which together constitute less than 0.5% of the total meteorite mass^{3,4}. One component of this Xe is enriched in the heavy Xe isotopes, and Anders *et al.* consider this to be evidence for the decay of a now extinct, fissioning superheavy element associated with these minor phases⁵.

In favourable circumstances, nuclear tracks produced by fission decay can be revealed by chemical etching or transmission electron microscopy. Flynn *et al.* have searched for tracks in chromite grains from Allende, and found none. They placed an upper limit on the *in situ* fission which is two orders of magnitude below that implied by the superheavy hypothesis⁶. Macdougall *et al.* examined an olivine adjacent to chromite in Murchison, selected from a separate which also contains the heavy Xe enrichment, and found no fission tracks⁷. These track studies, however, are inconclusive, since tracks in chromite and olivine might have been removed by a thermal event which would not have removed the Xe.

A more conclusive test of the superheavy fission hypothesis would be a search for fission related isotopic anomalies in other elements. These anomalies could be

less affected by thermal events than the Xe anomaly or fission tracks. Furthermore, isotopic measurements could also be made on the carbon and Q phases, which are not suitable for track studies.

In samples of bulk meteorites, the fission contributions to elements other than Xe and Kr are too small to be detected because of the greater natural abundances of the other elements in solid materials. If, however, the extreme enrichment of heavy Xe in these minor phases of meteorites is due to fission there should be corresponding enrichments of other fission products.

The light rare earth elements are particularly well suited for a search for these isotopic enrichments because: (1) the natural abundances of rare earths are low; (2) the fission yields of rare earths are high; (3) the techniques for accurate mass spectrometric measurement of rare earth isotopic ratios have recently been developed, and (4) the constancy of rare earth isotopic ratios, except where altered by nuclear processes, has been established.

The fission decay properties of U and the transuranic elements help predict which elements should exhibit the largest isotopic anomalies from the possible superheavy decay. The mass yield data for spontaneous or thermal neutron fission of ²³⁵U, ²³⁸U, ²³⁹Pu, ²⁴²Cm, and ²⁵²Cf all show a double peaked distribution with two rather broad maxima. The principal effect of an increase in mass of the fissioning nucleus is to shift the light mass peak to higher values, while the heavier mass peak remains essentially unchanged⁸. This behaviour, shown in Fig. 1, is explained by 50 proton and 82 neutron closed shell effects for nuclei on the heavy mass peak.

The heavy Xe yield data for Allende minor phases⁵, when normalised to ¹³⁶Xe = 6%, fits well on the left side of the high mass peak (Fig. 1). This suggests that if the heavy Xe is of fission origin the relative yields of Ba and the light rare earths, which are nearby in mass, should be of the same magnitude as for the thermal neutron fission of ²³⁵U, which has been well studied.

Using ²³⁵U mass yields, we can calculate the quantity of each rare earth and Ba isotope which would be produced by the amount of fission required to give the observed heavy

Fig. 1 Mass yield curves for thermal-neutron fission of ²³⁵U (—), ²³⁸U (---), ²⁴⁰Pu (---), and spontaneous fission of ²⁵²Cf (····) (figure from ref. 17). ■, Yields of ¹³⁶Xe attributed to fission from the Allende Xe rich phases, normalised to ¹³⁶Xe = 6% yield (data from ref. 3).

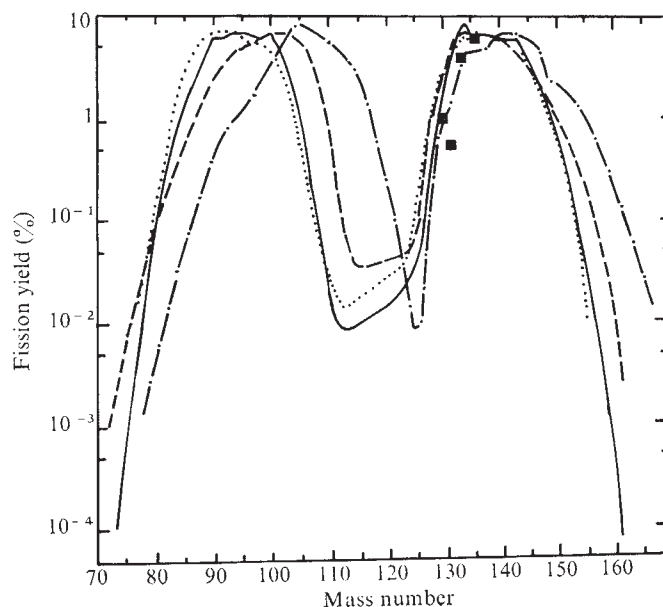


Table 1 Estimated fission induced deviations from normal isotopic ratios in the Allende Xe-rich phases

Isotope	Fission product*	$\delta(\text{‰})$ Q	$\delta(\text{‰})$ chromite	$\delta(\text{‰})$ carbon
¹³⁰ Ba	no	0	0	0
¹³² Ba	no	0	0	0
¹³⁴ Ba	no	0	0	0
¹³⁵ Ba	yes	+1.3	+0.25	+0.36
¹³⁶ Ba	no		(referenced isotope)	
¹³⁷ Ba	yes	+0.73	+0.14	+0.20
¹³⁸ Ba	yes	+0.11	+0.02	+0.03
¹³⁸ La	no	-0.73	-0.14	-0.20
¹³⁸ La	yes		(referenced isotope)	
¹³⁶ Ce	no	-2.2	-0.44	-0.62
¹³⁸ Ce	no	-2.2	-0.43	-0.61
¹⁴⁰ Ce	yes	-1.9	-0.35	-0.51
¹⁴² Ce	yes		(referenced isotope)	
¹⁴² Nd	no	-1.3	-0.26	-0.40
¹⁴³ Nd	yes	+1.5	+0.28	+0.39
¹⁴⁴ Nd	yes		(referenced isotope)	
¹⁴⁵ Nd	yes	+1.4	+0.25	+0.34
¹⁴⁶ Nd	yes	-0.30	-0.07	-0.09
¹⁴⁸ Nd	yes	+0.35	+0.08	+0.12
¹⁵⁰ Nd	yes	-0.66	-0.14	-0.22
¹⁴⁴ Sm	no	0	0	0
¹⁴⁷ Sm	yes	+2.5	+0.48	+0.67
¹⁴⁸ Sm	no		(referenced isotope)	
¹⁴⁹ Sm	yes	+1.3	+0.24	+0.34
¹⁵⁰ Sm	no	0	0	0
¹⁵² Sm	yes	+0.16	+0.04	+0.06
¹⁵⁴ Sm	yes	+0.16	+0.02	+0.02

*This column indicates whether fission contributes to the isotope in question. The estimated isotopic enrichments and depletions are based on the assumptions that the yield curve is the same as for ²³⁵U thermal neutron fission⁸, the natural abundances of the elements are average carbonaceous chondrite abundances¹⁵, and the natural isotopic ratios are terrestrial¹⁶. The quantities of fissiogenic ¹³⁸Xe in the Q, chromite, and carbon phases of Allende were taken as 3.7, 0.7, and 1.0×10^{-8} cm³ STP g⁻¹ (ref. 4).

Xe anomaly. Since the abundances of Ba and the rare earths in chromite, carbon, and Q from Allende have not been well established, we assume these abundances to be average chondritic and the natural isotopic ratios to be terrestrial. The deviations of the isotopic ratios from the terrestrial ratios due to the addition of fission products can then be estimated (Table 1). Several of these deviations are within the limits of detection reported by groups measuring the rare earths^{9,10} and Ba¹¹.

Measurement of isotopic anomalies in these elements would confirm the fission origin of the heavy Xe. Should the progenitor be a known transuranic element, and the anomalous Xe some form of fractionated fission Xe, it might be possible to match the rare earth enrichments to the measured yields for a transuranic.

Should the progenitor be a superheavy, we might expect some broadening of the heavy mass peak, as seen for ²⁵²Cf (Fig. 1), which would increase the yield of heavy rare earths. The yield of ¹⁵⁶Gd, for example, is 50 times greater from ²⁵²Cf fission than from ²³⁵U fission. Even larger amounts of ¹⁵⁶Gd might be expected from superheavy fission. Measurement of large isotopic anomalies in the heavy rare earths would suggest the progenitor was a superheavy. The absence of large isotopic anomalies in the heavy rare earths, however, would not eliminate the possibility of a superheavy progenitor. Ternary fission, which might not produce the heavy rare earths, has been suggested as a possible mode of fission decay for superheavy elements¹².

Other nuclear effects which might contribute to changes in the isotopic ratios are fission products from U and Pu,

neutron capture production, and spallation. A U abundance of 100 p.p.b. has been measured in the heavy Xe-rich Allende separate (J. Shirck, personal communication), while Anders *et al.* have measured 3 p.p.b. in a chromite-spinel fraction of the Xe-rich separate⁵. Even using the larger 100 p.p.b. value and a Pu/U ratio of 0.016 at formation¹³, the total contribution of U and Pu fission products over the 4.5×10^9 yr age of the meteorite is two orders of magnitude below the estimated superheavy contribution.

Neutron capture effects can be estimated from the ¹⁵⁰Sm/¹⁴⁹Sm ratio. Fission shielded ¹⁵⁰Sm is produced by a (n,γ) reaction on ¹⁴⁹Sm, which has the largest cross section of any Sm isotope. An upper limit on the neutron flux can already be set from the ⁸⁰Kr and ¹²⁹Xe measurements of Lewis *et al.*³.

Isotopic contributions from spallation can be estimated from the proton flux, derived from spallation contributions to Xe and Kr, and measurements of the abundances of the various spallation targets.

The actual abundances of the rare earths and Ba in chromite, carbon, and Q have not yet been well established. However, Anders *et al.* report abundances for several rare earth elements in an Allende chromite separate to be depleted by about a factor of 5 from chondritic⁵. This would increase the deviations from natural isotopic ratios in the chromite phase by a factor of 5 from those given in Table 1.

Despite the elusiveness of Q, it might prove to be the most suitable phase for isotopic measurements since: it is most enriched in the anomalous heavy Xe, and it is soluble in nitric acid, providing the means for separation of Q and its associated rare earths from the chromite and carbon phases.

The technique of measuring rare earth isotopic enrichment patterns has already proved valuable in identifying the ²³⁵U thermal neutron fission products in samples from the Oklo natural reactor¹⁴. Measurement of Ba and rare earth isotopic anomalies associated with the heavy Xe anomaly in carbonaceous chondrites could finally establish the fission origin of this heavy Xe. This technique would also be applicable to other samples where large concentrations of superheavy fission are expected.

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G. J. FLYNN
M. LOUBET*

McDonnell Center for the Space Sciences,
Washington University,
St Louis, Missouri 63130

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*Permanent address: Université Paris 6, Institut De Physique Du Globe 4 place Jussieu, 75230 Paris Cedex 05, France.

- Anders, E. & Heymann, D. *Science* **164**, 821-823 (1969).
- Dakowski, M. *EPSL* **6**, 152-154 (1969).
- Lewis, R., Srinivasan, B. & Anders, E. *Science* **190**, 1251-1262 (1975).
- Lewis, R., Gros, J. & Anders, E. *JGR* **82**, 5, 779-792 (1977).
- Anders, E., Higuchi, H., Gros, J., Takahashi, H. & Morgan, J. *Science* **190**, 1262-1271 (1975).
- Flynn, G., Fraundorf, P., Shirck, J. & Walker, R. in *Lunar Science VIII* 305-307 (The Lunar Science Institute, 1977).
- Maddougall, J. paper presented at Eighth Lunar Science Conference (1977).
- Hyde, E. *The Nuclear Properties of the Heavy Elements III: Fission Phenomena* (Prentice-Hall, Eaglewood Cliffs, 1964).
- Scheinin, N., Lugmair, G. & Marti, K. *Meteoritics* **11**, 357-358 (1976).
- Richard, P., Shimizu, N. & Allegre, C. J. *EPSL* **31**, 269-278 (1976).
- McCulloch, M., Papanastassiou, D. & Wasserburg, G. *Meteoritics* **11**, 331-332 (1976).
- Rao, M. *Nuclear Phys. A140*, 69-73 (1970).
- Droz, R., Morgan, C., Podosek, F., Poupeau, G., Shirck, J. & Taylor, G. *Astrophys. J.* **212**, 567-580 (1977).

- ¹⁴ Loubet, M. & Allegre, C. J. *Behavior of Rare-Earth Elements in the Oklo Reactor, GCA* (in the press).
¹⁵ Mason, B. *Handbook of Elemental Abundances in Meteorites* (Gordon and Breach, New York, 1971).
¹⁶ Friedlander, G., Kennedy, J. & Miller, J. *Nuclear and Radiochemistry*, Wiley, Chichester (1964).
¹⁷ Wahl, A. in *Physics and Chemistry of Fission* 1, 317–331 (IAEA, Vienna, 1965).

An early Cambrian chert biota and its implications

In the search for Precambrian life, much effort has been centred on the study of stromatolitic cherts and their predominantly blue-green algal flora¹. Nonetheless, it is common for reviews of evolution of the biosphere to switch from consideration of Pre-Vendian chert floras to Vendian soft bodied or trace fossils and thence to Tommotian shelled invertebrates². A more objective approach will involve tracing organic development across the Cryptozoic–Phanerozoic transition from within a single habitat. Until this has been attempted, our views of changing biotas will be obscured by the changing facies patterns. I report here the discovery of a new early Cambrian flora and fauna from a typically 'Precambrian' habitat, a chertified oncolite association.

The chert horizon occurs within parallel laminated dolomites about 10 m above the base of the Eilean Dubh Formation of the Durness Group of north-west Scotland, near to Inchnadamp (NC 253213). Except for an unverified report of *Salterella*³ this is the first report of organisms from this predominantly dolomitic formation and confirms the early Cambrian age of the lower part. The underlying strata contain shelly fossils typical of Lower Cambrian rocks while overlying horizons within the Durness Group are of early Ordovician age³.

The complex diagenetic history of these Durness dolomites⁴ is evident from the varying degrees of organic and skeletal preservation in the Inchnadamp chert. Invertebrate skeletal remains, preserved in silicified dolomite clasts, have suffered much recrystallisation before silicification. Preliminary identifications of rare fragments show the presence of trilobites, hyolithids (Fig. 1a), sponge spicules and archaeocyathids (Ajacicyathacea). The presence of *in situ* invertebrates is indicated by pellet-filled burrows in the associated laminated dolomites.

Algal participation in carbonate accretion is shown by the abundant filamentous and coccoid structures preserved as kerogenous or pyritic material in oolites and oncolites (Fig. 1). Unbranched, septate filaments of 2–5 µm diameter occur as envelopes around oolite laminae and in the outer layers of oncolites, mostly lying normal to the surface (Fig. 1a, f). These closely resemble recent and fossil filamentous blue-green algae from similar facies^{5,6} and the mucilage probably contributed to oolite growth.

Branched filaments 1–3 µm in diameter occur as small, isolated and erect tufts up to 25 µm across, preserved in dark pyritic material (Fig. 1b, d). Their form and oncolithic habit compare closely with *Corbularia conglutinata* Vologdin from the Lower Cambrian of Asia⁷. The small cell diameter suggests cyanophyte affinities.

The problematic organism *Renalcis* is excellently preserved in several oncolites, revealing forms comparable with *R. levis* Vologdin (Fig. 1c). This material is clearly not foraminiferid and resembles colonies of coccoid cyanophytes embedded in a gelatinous envelope (compare with *Anacystis*). I have previously argued in conjunction with Riding⁸ that the 'chamber walls' of *Renalcis* cannot represent the single cell wall of blue-green alga, and here they are seen to consist of a chain of minute coccoid cells about 2 µm in diameter, their packing reduced towards the central 'chamber'. This, and evidence of shrinkage cracks in other material, leads one to support the blue-green algal interpretations of Korde⁹ and Hofmann¹⁰. Nevertheless it is pos-

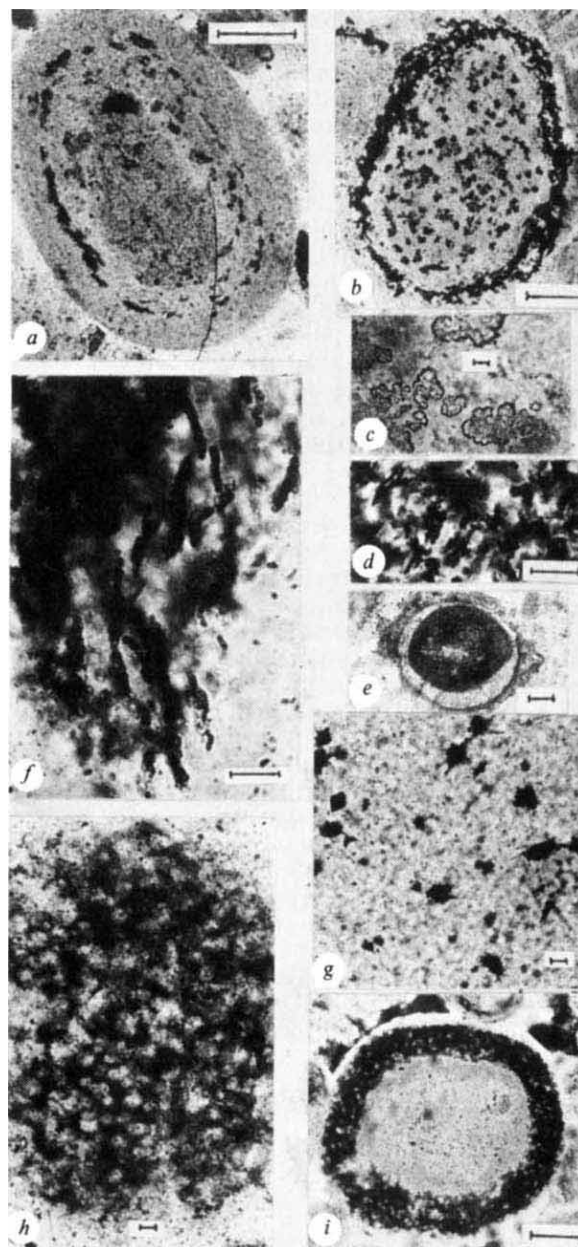


Fig. 1 Organic structures from the Lower Cambrian Inchnadamp Chert, Eilean Dubh Formation, N.W. Scotland. *a*, Oolite enclosing algal thalli; *b*, oncolite with branched algal filaments (*Corbularia* sp.); *c*, *Renalcis* sp. showing coccoid wall structure; *d*, detail of *Corbularia* filaments; *e*, hyolithid shell; *f*, blue-green algal filaments; *g*, acanthomorph acritarchs (*Micrhystridium* sp.); *h*, coccoid (eukaryotic?) cells; *i*, similar forming an oncolithic envelope around a clast. Scale bar, 100 µm in *a*, *b*, *e* and *i*; 10 µm in *c*, *d*, *f*, *g* and *h*.

sible that *Renalcis* is an artificial and polygot genus, encompassing other algal and protistan groups.

Larger coccoid cells 12–25 µm in diameter are probably eukaryotic (Chlorophyta?). These occur singly or as colonies coating the outer margins of round carbonate clasts (Fig. 1h, i) and occur throughout some oncolites. Cell walls and contents are not preserved, their form merely being outlined by strongly pigmented material that was once a mucilaginous matrix (compare ref. 10). Acanthomorph acritarchs 12–20 µm in diameter (*Micrhystridium* sp.) are evenly distributed through a few silicified dolomite clasts (Fig. 1g) where they are associated with archaeocyathid fragments.

The scarce invertebrate remains, acritarchs and *Renalcis* suggest that sediments were deposited in an offshore facies,

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*Department of Geology,
University of Hull,
Cottingham Road, Hull, UK*

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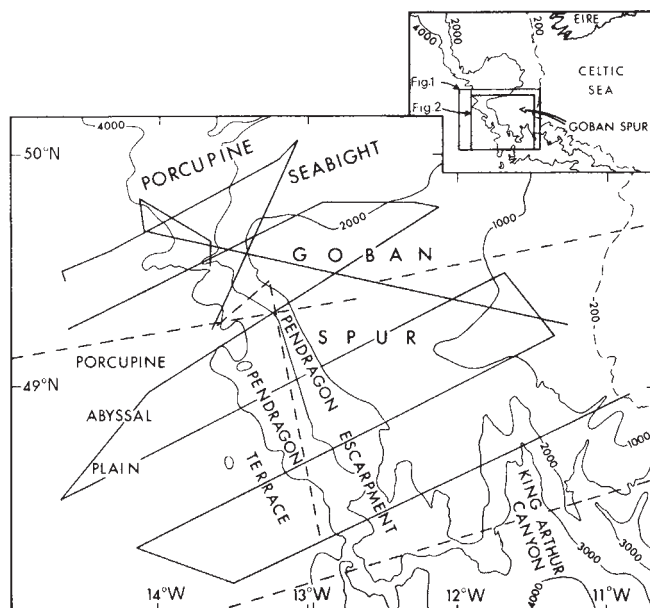
- ¹ Schopf, J. W. *Proc. N. Am. Paleontol. Conv.* 1013–1057 (1969).
- ² Cloud, P. *Paleobiology* 2, 351–387 (1976).
- ³ Cowie, J. W. in *Cambrian of the British Isles, Norden and Spitzbergen* (ed. Holland, C. H.) 123–155 (Wiley, London, 1974).
- ⁴ Swett, K. J. *sedim. Petrol.* 35, 928–935 (1965).
- ⁵ Bathurst, R. G. C. *Carbonate Sediments and Their Diagenesis*, 77–91, 295–319 (Elsevier, New York, 1971).
- ⁶ Schopf, J. W., Ford, T. D. & Breed, W. J. *Science* 179, 1319–1321 (1973).
- ⁷ Luchinina, V. A. *Akad. Nauk SSSR. Trud.* 216, 1–99 (1975).
- ⁸ Riding, R. & Brasier, M. *Nature* 257, 208–210 (1975).
- ⁹ Korde, K. B. *Akad. Nauk SSSR. Trud.* 89, 1–147 (1961).
- ¹⁰ Hofmann, H. J. *Am. J. Sci.* 275, 1121–1132 (1975).
- ¹¹ Downie, C. *Rev. Palaeobot. Palynol.* 18, 57–60 (1974).
- ¹² Garrett, P. *Science* 169, 171–173 (1970).
- ¹³ Riding, R. *Paleontology* 20, 33–46 (1977).
- ¹⁴ Stanley, S. M. *Proc. nat. Acad. Sci.* 70, 1486–1489 (1973).
- ¹⁵ Conner, D. *Proc. 2nd Int. Coral Reef Symp.* 1, 370 (1974).

Continental margin fault pattern mapped south-west of Ireland

A SIMPLE model for continental basement structures at rifted continental margins comprises large fault blocks which trend approximately parallel to, and step down towards, the continental-ocean boundary (for example, see ref. 1). These blocks may be cut by faults which strike across the margin, and, in many theoretical discussions, are shown as being separated from the true oceanic crust by an intermediate zone (see transitional crust of Fig. 3, ref. 2). On many rifted margins these features are deeply buried by

Goban Spur is separated from the continental shelf and the Pendragon Terrace by large boundary discontinuities, presumed to be faults (inner boundary fault and Pendragon Discontinuity respectively). The amplitudes of these displacements are about 2.0 and 1.5 s double time respectively, and although the latter is not particularly large it does coincide with a change in basement characteristics. Our

Fig. 1 Track chart of seismic lines occupied by RRS Shackleton over Goban Spur during cruises 5/76 and 4/77 (— — —, bathymetry only). Bathymetry in m after various sources. Inset shows location of Figs 1 and 2.



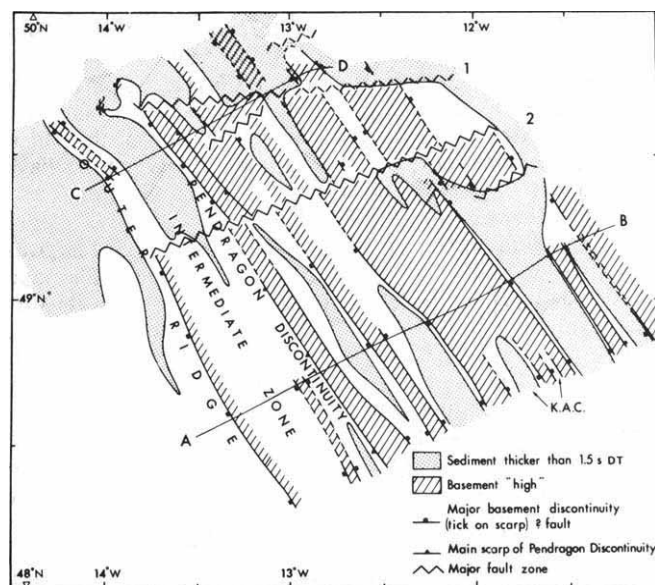


Fig. 2 Summary structural map of Goban Spur and adjacent areas, K.A.C., King Arthur Canyon. Sections AB and CD are shown in Fig. 3.

gravity data indicate that it also marks the point of maximum change in thickness of the crust. Where the Pendragon Discontinuity acts as a sediment dam it underlies a prominent bathymetric feature called Pendragon Escarpment, but in the south, the escarpment lies about 10 km west, over a subsidiary ridge.

The Pendragon Discontinuity separates Goban Spur proper from the intermediate zone. Here, basement morphology is relatively subdued (0.5 s double time in amplitude), and generally lies about 1.0 s double time deeper than to the east. The basement of the intermediate zone south of fault zone 2 is covered by a relatively thin sedimentary sequence, and although we have no sample evidence for its composition, geomagnetic characteristics suggest that it is largely similar to that beneath the spur. A large magnetic anomaly occurs, however, over the outer part of Pendragon Terrace, indicating the presence of basic igneous rocks. An outer ridge (Fig. 2) separates the intermediate zone from oceanic crust under the Porcupine Abyssal Plain, where basement morphology is rough but of low amplitude. The depth to oceanic basement is about 7.0 s double time and the sediment cover is relatively thin. Linear magnetic anomalies resulting from regular seafloor spreading are not well developed until 14°30'W is reached, although the magnetic field is rough and indicative of oceanic basement. We tentatively identify the western flank of the outer ridge as the location of the continent-ocean boundary.

Having briefly described the tripartite division of the margin structure where it is most clearly developed in the south, we now consider the northern part of Goban Spur, where the fracture pattern is crossed by fault zones 1 and 2 (Fig. 2). Fault zone 2 is a complex structure. Beneath Goban Spur it seems to act as a scissor fault, throwing the crest of the Pendragon Discontinuity up to the north, but the general level of basement to the east of the discontinuity down to the north. The fault also causes an alteration in the strike of the Pendragon Discontinuity (making it more westerly), coincides with an increase in amplitude of the discontinuity to about 4.0 s double time, and displaces it slightly in a dextral sense. Beneath Pendragon Terrace, however, zone 2 appears to act as a simple fault throwing down to the north. The depth to basement in the intermediate zone increases from about 5.5 s to about 7.3 s

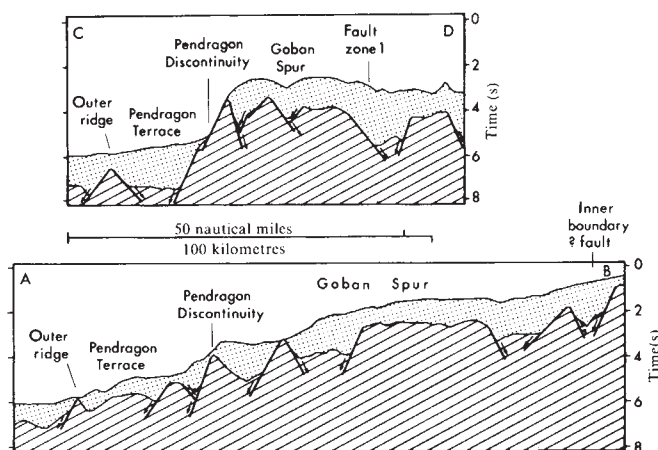
double time across the fault. Evidence that fault zone 2 has influenced the development of oceanic basement under the nearby parts of Porcupine Abyssal Plain comes from magnetic anomaly data, which show that sea-floor spreading anomalies are better developed to the north of the fault. An increase in sediment thickness in the abyssal plain is also observed across the westward extension of fault zone 2. Despite these complexities we can confidently recognise the tripartite subdivision of the continental margin between fault zones 1 and 2.

Fault zone 1 constitutes the northern structural boundary of Goban Spur. To the north of this, basement highs and lows are down-faulted into the southern part of Porcupine Seabight, so that the general level of basement is 2–3 s double time deeper. The thickness of the sedimentary sequence increases rapidly across the fault zone. Although the general structural pattern of Goban Spur can be traced into Porcupine Seabight, it is not possible to follow individual features, such as the Pendragon Discontinuity, and hence we do not know if there is any strike-slip component of motion on fault zone 1. The horst and graben style of tectonics identified beneath Porcupine Seabight has approximately the same trend as that beneath Goban Spur and may owe its existence to the same phase of rifting.

We believe that we have mapped a continental margin fracture pattern some 150 km wide that was initiated during the rifting phase that preceded the opening of the North Atlantic Ocean at this latitude. The trend of the basement horsts and grabens is parallel to the sea-floor spreading magnetic anomalies observed further west⁶. On the evidence of these anomalies, the opening of the North Atlantic hereabouts is thought to have begun in Upper Cretaceous times. This would suggest that the rifting phase is of Cretaceous age. Our records show that there has been little or no vertical movement between adjacent fault blocks since the rifting phase. Thus, the subsequent history of the margin is one of overall limited subsidence. There is good evidence for Goban Spur being underlain by Hercynian granites^{3,7}, whose relative buoyancy in the crust could account for the lack of subsidence and thin sediment cover. Fault zones 1 and 2 are approximately parallel to the Hercynian structural grain projected from areas surrounding the Celtic Sea. Apparently, the rifting tectonics have been modified by reactivation of these old trends to produce the observed fracture pattern.

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Fig. 3 Cross sections across Goban Spur replotted from seismic reflection profiles. Vertical scale is in seconds double time. See Fig. 2 for locations.



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RICHARD V. DINGLE

Department of Geology,
University of Cape Town,
Rondebosch,
South Africa

ROGER A. SCRUTTON

Grant Institute of Geology,
University of Edinburgh,
West Mains Road,
Edinburgh, UK

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¹ Falvey, D. A. *Aust. petrol. Exp. Ass.* 14, 95–106 (1974).

² Dewey, J. F. & Bird, J. M. *J. geophys. Res.* 75, 2625–2647 (1970).

³ Pautot, G., Renard, V., Auffret, G. & Pastouret, L. *Nature* 263, 669–672 (1976).

⁴ Scrutton, R. A., Stacey, A. P. & Gray, F. *Earth planet. Sci. Lett.* 11, 140–146 (1971).

⁵ Smith, S. G. & van Riessen, E. D. *Mar. geophys. Res.* 2, 83–94 (1973).

⁶ Williams, C. A. *Earth planet. Sci. Lett.* 24, 440–456 (1975).

⁷ Day, G. A. & Williams, C. A. *Earth planet. Sci. Lett.* 8, 205–214 (1970).

Holocene variations in the level of Lake Bosumtwi, Ghana

RECENT surveys of the changing climate of Africa during the Late Quaternary have shown that although a reasonably well established climatic sequence is now available for some parts of the continent, there exist very large areas which have yet to yield any well-dated, unequivocal palaeoclimatic data^{1–3}. Most notable among the latter are the lowland tropical forest regions. Despite the absence of information from such critical areas, attempts have been made to reconstruct atmospheric conditions over West Africa during the Late Quaternary^{2,4,5}, but obviously no uniformly satisfactory models can be produced until these data gaps have been filled. Study of changing lake levels has proved a particularly valuable palaeoclimatic tool⁶ and we report here the first ¹⁴C dates of former levels of a lake that lies within the present lowland forest zone of West Africa. Our work

indicates that lakes of the humid tropics can provide valuable palaeoclimatological information that may be less apparent from the study of arid zone lakes. In particular, we present evidence suggesting that major regressions of equatorial African lakes during the Holocene, rather than being a consequence of exceptional aridity, may have occurred during periods of slightly higher temperatures and greater evaporation.

Lake Bosumtwi lies within a steep-sided crater that was produced by meteoritic impact 1.3 ± 0.3 Myr ago⁶. The lake has a maximum depth of about 80 m, and in 1976 its surface lay at +100 m OD, some 105 m below the lowest point in the surrounding rim of hills and 100–200 m lower than the land outside the crater. Maintenance of the lake is thus largely dependent upon rainfall over the crater, a catchment of about 400 km². Mean annual precipitation is 1,520 mm and temperature 26 °C.

A clearly defined wave-cut bench encircles the lake at about 110 m above present lake level and merges into the lower slopes of the valley that provides the lowest point of exit from the crater (Fig. 1). This, the only valley to cut cleanly through the crater rim, has obviously functioned as an overflow channel in the past. No material suitable for dating has been found here, nor on the bench, all the dated samples coming from lacustrine sediments exposed within +25 m of the present lake surface.

Sample sites are shown on Fig. 1; samples and their ¹⁴C ages are summarised in Table 1. On the basis of these results, plus historical records^{7,8} and data from the Geological Survey Department's gauge at Apewu (1933 onwards), an attempt has been made to reconstruct the behaviour of Lake Bosumtwi over the past 10,000 yr (Fig. 2). Sediments older than the turbidite silts (Table 1) have not been identified⁹. This is somewhat surprising in view of the relative antiquity of the lake, particularly as the strong development of the high bench and overflow channel testify to one or more prolonged periods of maximum lake level.

The Holocene behaviour of Lake Bosumtwi shows some similarities with lakes elsewhere in West and East Africa. Generally high levels are known to have occurred throughout the tropics during the early Holocene^{2,3}. In the case of lakes that occupied basins in what are now arid or semi-arid regions, the climate must have been markedly wetter than now. Even though Bosumtwi was also high at this time it cannot be demonstrated that precipitation here was necessarily greater than at present. The lake has been rising continuously for at least the past 120 yr (Fig. 2), so prevailing climatic conditions seem adequate to maintain a very high level. Indeed, if evaporation from the lake surface is taken to be about 1.45 m yr^{-1} (ref. 10) (mean annual pan evaporation at Kumasi, 30 km away, was 1.4 m over the period 1954–1974), then a simple mass balance calculation is sufficient to show that the present hydrological regime is capable of sustaining the lake at overflow level. It is thus possible that around 9,000–10,000 yr BP the climate was essentially similar to today. Abundant leaf impressions preserved in the turbidites indicate that although the forest flora may have differed slightly in composition from modern local forests, the climate of the early Holocene must have been comparable with the present¹¹.

Unfortunately, we have been unable to obtain dateable material from the upper part of the turbidite silts and so have no precise idea how long the very high stand persisted. The considerable thickness (~35 m) of silts above the dated horizons at Banson suggests that it continued well into the Holocene. The regression that followed clearly involved a considerable fall in lake level, the resulting erosion surface having been identified at elevations ranging from +2 m to +18 m. The subsequent transgression was coeval with positive changes in level that are known to have occurred in lakes throughout tropical Africa around 2,000 yr BP–3,000 yr BP (refs 2 and 3).

Fig. 1 Map of Lake Bosumtwi and inner part of crater, showing location of samples detailed in Table 1. Solid band below 250 m is erosional bench at level of overflow channel.

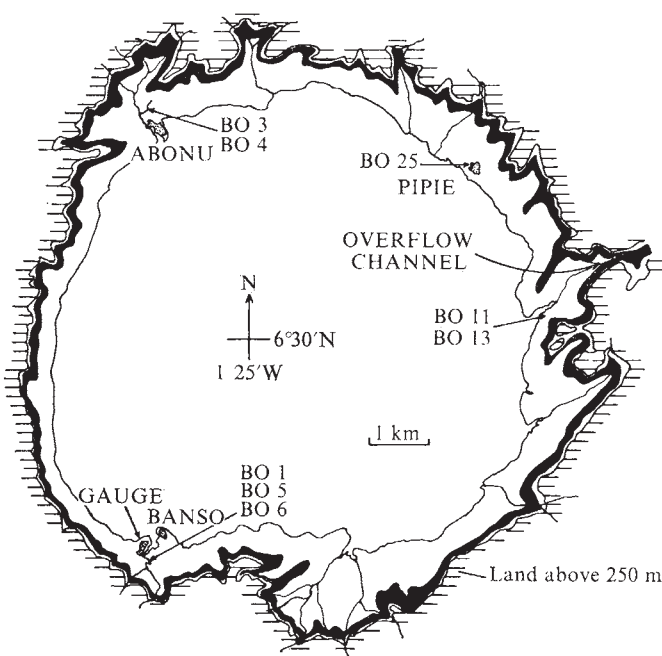


Table 1 Summary of samples* with ^{14}C ages

Sample no. locality	Lab. no.	Nature of sample	Geological context	State of lake	^{14}C age (yr b.p.)
B01 Banso	GIF — 3650	Wood	Turbidite silts	Very high > +35 m	9880±220
B05 Banso	GIF — 3991	Wood	Turbidite silts	Wood accumulated in deep anoxic waters of a stratified lake	10000±220
B03 Abonu	GIF — 3651	<i>M. tuberculata</i>	Deltaic sands	Moderately high ~ +23 m	2520±200
B04 Abonu	GIF — 3652	<i>M. tuberculata</i>	Deltaic sands	Moderately high ~ +25 m	1940±300
B06 Banso	GIF — 3992	Wood	Root penetrating turbidite silts	Rising, vegetation killed by transgression	3620±110
B013 Konkoma	GIF — 3996	Wood	Remnants of vegetation that colonised exposed turbidite silts		3020±110
B011 Konkoma	GIF — 3995	<i>M. tuberculata</i>	Deltaic sands	Moderately high ~ +18 m	700±90
B025 Pipie	GIF — 3998	<i>M. tuberculata</i>	Silt between granite boulders	Moderately high > +19 m	1650±110

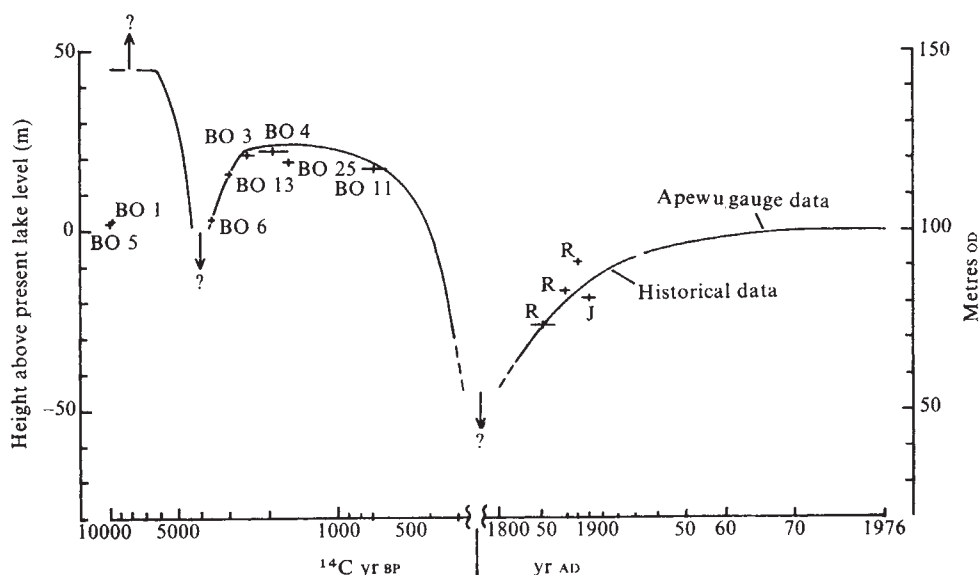
*Used in preparation of pre-nineteenth century section of Fig. 2. For precise sample localities see Fig.1.

Historical records^{7,8} and the oral traditions of fishermen living around the lake are consistent in indicating that 200–300 yr ago Bosumtwi was very low indeed. The geological evidence for moderately high levels persisting until around 700±90 yr BP implies, therefore, that a rather abrupt regression must have started in the thirteenth or fourteenth century. In attempting to understand the regression of Bosumtwi, it is helpful to compare the behaviour of the lake with trends established for other climatic zones in West Africa, comparative palaeoclimatic data from elsewhere in the forest zone being nonexistent. Over the period of systematic observation, variations in the rate of rise of Bosumtwi have closely matched variations in the precipitation of the Sahel¹² (Fig. 3). Although climatic oscillations in the two regions may not always have progressed in unison (ref. 13 and J. Maley personal communication), the Sahel can provide a particularly sensitive indication of changes in the precipitation brought by the monsoon. Figure 3 shows, for example, that the Sahelian drought of the late 1960s and early 1970s was clearly reflected in Lake Bosumtwi by a period of lower than average rises. The lake, however, continued to rise, showing that this region of the forest was not exposed to true drought. Obviously here regression is only to be expected when monsoonal precipitation is drastically reduced and the Sahel subject to prolonged and exceptional aridity. This observation is of considerable importance in understanding the behaviour of Lake Bosumtwi in the more distant past.

Looking first at the period between 700 yr BP and the present, there is evidence to suggest that during the seventeenth and eighteenth centuries parts of the Sahel were slightly moister than at present¹⁴, while there was at least abundant rainfall in the Chari-Logone catchment with which to maintain Lake Chad at very high levels¹⁵. At the same time, by analogy with the twentieth century, Lake Bosumtwi should have been very high or rising rapidly. The latter would certainly be consistent with oral traditions, which although indicating low lake levels, also abound with references to lake shore villages being abandoned because of inundation^{7,8}. Thus the last regression must have occurred between the thirteenth and sixteenth centuries.

Over the last 1,000 yr Lake Chad is known to have oscillated considerably in level. A number of regressions are recognisable, including one which started between the twelfth and thirteenth century^{4,13} and may, therefore, have been synchronous with the last fall of Lake Bosumtwi. None of these low stands of Lake Chad, however, seem to have been of long duration, the latest accompanying the recent drought and the last three occurring within the past 150–175 yr (ref. 15). Throughout this period, Bosumtwi has risen continuously, so monsoonal precipitation in the forest zone can clearly never have been greatly reduced during the nineteenth and twentieth centuries, despite decreases on a scale sufficient to allow Lake Chad to fall. As the twelfth to thirteenth century regression of Lake Chad was of apparently comparable magnitude with these more recent

Fig. 2 Holocene fluctuations in level of Lake Bosumtwi (BO). Curve constructed from: (1) ^{14}C dates of samples described in Table 1; (2) historical reports of changes in lake level during the nineteenth and early twentieth century; (3) Apewu gauge data (1934 to present). Horizontal bars represent error on dates (standard error for ^{14}C ; estimated range for historical dates). R, dates from ref. 9; J, date from ref. 8. Note log time scale.



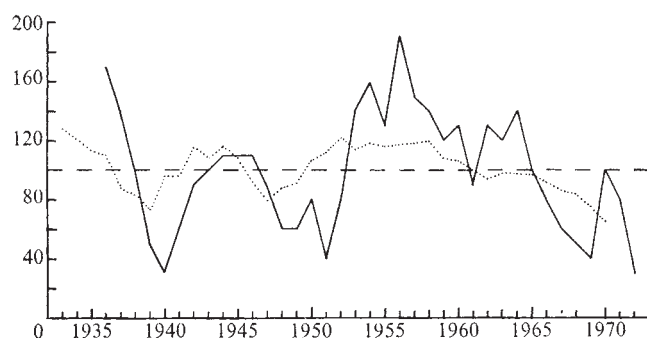


Fig. 3 Five-year running means of monsoon rainfall in the Sahel zone (from ref. 16, Fig. 2) and increments in the level of Lake Bosumtwi (from Apewu gauge record), both expressed as percentage deviation from the mean. . . . Sahel; — Lake Bosumtwi.

falls, there seems no reason to suspect an exceptionally different rainfall regime at that time, either. The abrupt decline of Lake Bosumtwi is unlikely, therefore, to have been the result of drastically lower rainfall, but was more probably due to the combined effects of a rise in temperature and accompanying slight decrease in precipitation inducing evaporation losses to exceed water input. An inverse relationship has been demonstrated to exist between temperature and precipitation in some parts of tropical Africa^{4,16}. In the Bosumtwi region, during the 1967–1974 dry period, annual precipitation was 92% of the 1932–1974 mean, but temperatures were around 0.2 °C higher than the long-term average. If 1967–1974 conditions were to become the norm, a further rise in mean annual temperature of only 0.5 °C might be sufficient to cause the transition to a negative hydrological balance¹⁰. As such a rise would probably be accompanied by an even greater decrease in rainfall, it is obvious that only a slight deviation from climatic conditions that have prevailed at times during the twentieth century would be necessary to initiate a regression of Lake Bosumtwi.

Evidence of elevated tropical temperatures in the twelfth and thirteenth centuries should not be altogether unexpected. This was a period of maximum solar activity¹⁷, and there are abundant indications of notable warmth in the temperate northern hemisphere during late Mediaeval times^{18–20}. Records of much reduced discharge of the Nile, which must in part have been due to lowering of the great lakes of East Africa^{21,22}, are perhaps further testimony to accelerated evaporation in the equatorial zone at that time, while climatically-induced environmental stress during the thirteenth and fourteenth centuries may also have been responsible for the rather sudden migration of pastoralists from the Bahr-el-Ghazal region of the Sudan southwards into Uganda²³. Lamb¹⁶ has observed that the conjunction of lowered equatorial rainfall with periods of increased temperature, and thus greater moisture input into the atmosphere, must imply significant transport of moisture out of the equatorial zone. Such a process could have been particularly intense during the late Mediaeval thermal optimum, when poleward displacement of moist tropical air may have helped bring about the widely reported exceptional precipitation that affected higher latitudes at that time^{18–20}.

Identifying the cause of the mid-Holocene fall of Lake Bosumtwi must await more precise dating of this event. It was probably coeval with the general decline of African lakes, Saharan, Sahelian and equatorial, which commenced between 6,000 yr BP and 5,000 yr BP^{1–3}. Diverse sorts of evidence indicate that the period 6,000 yr BP–4,000 yr BP was everywhere characterised by relative warmth^{20,24,29}, so elevated evaporation rates are likely to have played an

important part in the fall of Lake Bosumtwi at that time as well.

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M. R. TALBOT

Department of Earth Sciences,
University of Leeds,
Leeds, UK

G. DELIBRIAS

Centre des Faibles Radioactivités,
CNRS-CEA,
B.P. 1,
91190 Gif-sur-Yvette, France

Received 11 May; accepted 30 June 1977.

- 1 Livingstone, D. A. *Ann. Rev. ecol. Systematics* 6, 249–280 (1975).
- 2 Rognon, P. *Rev. Geogr. phys. Geol. dyn.* 18, 251–282 (1976).
- 3 Street, F. A. & Grove, A. T. *Nature* 261, 385–390 (1976).
- 4 Maley, J. *Palaeogeog. Palaeoclimatol. Palaeoecol.* 14, 193–227 (1973).
- 5 Maley, J. *C.R. Acad. Sci. Paris Ser. D* 283, 337–340 (1976).
- 6 Schnetzler, C. C., Pinson, W. H. & Hurley, P. M. *Science* 151, 817–819 (1966).
- 7 Junner, N. R. *Gold Coast geol. Surv. Bull.* 8 (1937).
- 8 Rattray, R. S. *Ashanti* (Clarendon, Oxford, 1923).
- 9 Talbot, M. R. *20th ann. Rep. res. Inst. Afr. Geol.* 69–73 (University, Leeds, 1976).
- 10 Langbein, W. B. *U.S. Geol. Surv. Prof. Paper* 412 (1961).
- 11 Hall, J. B., Swaine, M. D. & Talbot, M. R. *Palaeogeog. Palaeoclimatol. Palaeoecol.* (in the press).
- 12 Winstanely, D. *Nature* 245, 190–194 (1973).
- 13 Maley, J. in *Recherches Françaises sur le Quaternaire hors de France* (in the press).
- 14 Nicholson, S. E. thesis, Univ. Wisconsin, 1976).
- 15 Maley, J. *Palaeoecol. Afr.* 9, 44–47 (1976).
- 16 Lamb, H. H. *Geogr. J.* 132, 183–212 (1966).
- 17 Suess, H. E. *Palaeogeog. Palaeoclimatol. Palaeoecol.* 10, 199–202 (1971).
- 18 Lamb, H. H. *Palaeogeog. Palaeoclimatol. Palaeoecol.* 1, 13–37 (1965).
- 19 Ladurie, E. le R. *Times of Feast, Times of Famine* (George Allen & Unwin, London, 1972).
- 20 LaMarche, V. C. *Science* 183, 1043–1048 (1974).
- 21 Brooks, C. E. *Experientia* 10, 153–192 (1954).
- 22 Dale, I. R. *Emp. Forest. Rev.* 33, 23–29 (1954).
- 23 Posnansky, M. in *Geographical Papers in Honour of K. C. Edwards* 215–223 (University, Nottingham, 1970).
- 24 Hammen, T. van der, Maarleveld, G. C., Vogel, J. C. & Zagwijn, W. H. *Geol. Mijnb.* 45, 79–95 (1967).
- 25 Wollin, G., Ericson, D. B. & Fwing, M. in *Late Cenozoic Glacial Ages* 199–214 (ed. Turekian, K. K.) (Yale, New Haven, 1971).
- 26 Castet, R. W., Adams, D. P. & Sims, J. D. *Quat. Res.* 7, 133–143 (1977).
- 27 Dansgaard, W., Johnsen, S. J., Clausen, H. B. & Langway, C. C. in *Late Cenozoic Glacial Ages* 37–56 (ed. Turekian, K. K.) (Yale, New Haven, 1971).
- 28 Möner, N.-A. & Wallin, B. *Palaeogeog. Palaeoclimatol. Palaeoecol.* 21, 113–138 (1977).
- 29 Zinderen Bakker, E. M. van & Coetzee, J. A. *Palaeoecol. Afr.* 7, 151–181 (1972).

Final results of study of infants at risk of sudden death

IN 1974 we described how we had used routine obstetric and perinatal records to construct a scoring system for identifying within 24 h of birth infants at risk of dying unexpectedly, and we reported the results of the first year of a prospective evaluation of the system¹. We now have the final results of the study which confirm that the scoring system is effective. The study data and subsequent work suggest that deaths can be reduced by increased surveillance of high risk infants.

The scoring system used combined observations of eight variables²—mother's age and blood group, whether she was reported as having a urinary infection or polyhydramnios during pregnancy, the duration of the second stage of labour, the birth order of the infant, whether it was premature, and whether the mother intended to breast feed. Increasing age of mother, blood group A and breast feeding were associated with low risk. The high risk infants were not identified by single factors as is the common practice^{3–5} but by the weighted combination of all observations.

The organisation of the prospective study⁶ was briefly as follows. Each week day the score of each new baby was calculated. Babies born at home were included. Infants scoring above the estimated 85th quantile were designated high risk. After excluding high risk infants with gross congenital anomalies, the high risk infants were randomly

allocated to 'observation' or control groups. At certain times because of holidays, all high risk infants were allocated to the control group. The observation group were invited to participate in the study. This comprised a clinical examination made within 48 h of birth, a second at 5 weeks, together with 10 visits to the home spread over the first 20 weeks of life by specially appointed health visitors. Half of the infants who dropped out of the follow-up study are included in the study group and half with the refused. All babies born in 1973 and 1974 in the County Borough of Sheffield, as defined before the local government reorganisation, were included in the prospective study. By the end of 1976 all these infants were at least 2 yr old, the generally accepted upper age limit for the occurrence of unexpected infant death⁷.

The results are summarised in Table 1. A total of 11,424 infants were scored. Our count for 1973 differs by 6 from that published by the Office of Population Censuses and Surveys. Due to local government reorganisation no official figures are available for the study area for 1974, but our study continued unaltered and we think few if any babies were not scored. In all 15.7% of infants were scored as high risk.

Table 1 Post perinatal infant mortality, that is, 1 week—1 year, in various groups of the prospective study.

	No. in group	Probably unpreventable deaths	Unexpected deaths No.	% of group
High risk				
Observed	627	1	2	3.2
Control	922	4	9	9.8
Refused	210	—	3	14.3
Excluded	35	12	1	—
Low risk	9,630	12	15	1.6
Total	11,424	29	30	2.6

Deaths are shown under two headings, probably unpreventable and unexpected deaths⁸. Post-mortem assessments were made without knowledge of the high risk grouping. Deaths due to anomalies, for example congenital heart defect, tumour or Werdnig-Hoffman syndrome together with deaths of infants who never left hospital are shown as probably unpreventable. One high risk infant excluded and kept in hospital several weeks because of a suspected congenital heart defect, died unexpectedly a week after discharge, but the anomaly could not be demonstrated post mortem. Table 1 shows that the unexpected death rate in the high risk control group is more than 6 times that of the low risk group (relative risk 6.3). This difference is unlikely to occur by chance ($P < 0.001$), and shows that the scoring system is highly effective. The relative risk of unexpected death in the group that refused is 9.2 and is also statistically significantly greater than 1, $P < 0.001$. The relative risk of the observed group is only twice that of the low risk group.

Between the age of 20 weeks and 1 yr there were three unexpected deaths, one in the high risk control group and two in the low risk group. In addition, there were five unexpected deaths between the ages of 1 and 2 yr, one in each of the first three high risk groups and two in the low risk group. The results are therefore essentially the same if the data are examined at 20 weeks, at age 1 yr as in Table 1, or at 2 yr. Overall these final results are remarkably similar to those previously reported¹.

The possibility that surveillance reduces mortality requires comment. The difference between the unexpected death rate in the studied group and the high risk control group is not statistically significant using a χ^2 test. If we group those who refused surveillance with the controls, the chance of a difference as large or larger, using an exact one-sided test of significance, is 0.076.

Our primary objective was to observe the high risk group, but to ignore serious medical conditions would have been

unethical. By special arrangement our team of visitors were allowed to take infants directly to hospital if necessary and when the 5 week clinical examination showed that bottle feeds were often over-concentrated⁹ active steps were taken to ensure that feeds were correctly made up. It is possible to dismiss the reduction in the unexpected death rate in the study group as non-significant, as Magura has done¹⁰, but the alternative hypothesis that the death rate is reduced is more likely.

The difference between study and control groups is also less different than might be expected because all groups benefited from the study, for example, the campaign to explain the dangers of overstrength feeding was not confined to the study group. The benefit is reflected in a reduction in the post perinatal mortality rate. For 1968–1972 the average post perinatal infant mortality rate for Sheffield was 8.2 per thousand, compared with a rate of 7.6 per thousand in England and Wales in this period. For the study period the rate was 5.2 per thousand—England and Wales 7.4. Had the observed group had the same mortality rate as the control group, the rate would have been 5.7 per thousand. On the other hand, if the benefits of observation were extended to all high risk infants a mortality rate of 4.8 per thousand might be expected.

Funds did not permit the prolongation of the study to enable us to establish unequivocally the benefits of surveillance or to determine which components reduce mortality. But, since January 1975 all infants born in the new County District of Sheffield have been scored and the community health authorities, including the health visitors, have been regularly notified of all high risk infants. In addition since August 1975 a new system has been introduced whereby all infants are examined in the home by a health visitor at the age of 4 weeks. The post perinatal infant mortality rate for 1975 and 1976 is 4.7 per thousand, which is almost exactly the rate expected if surveillance is effective, and is statistically significantly lower than 6.3 per thousand, the post perinatal mortality rate for these years in England and Wales, $P < 0.01$.

We conclude that infants at increased risk of unexpected post perinatal death can be identified at birth. Our data also suggest that post perinatal deaths may be prevented by alerting the primary health care nurses to the surveillance of this group. We have also recently shown that the identification of high risk children can be made more effective by incorporating information on the early development of the child by a multi-stage scoring system. In a small prospective test 71% of unexpected deaths were allocated to the high risk group which had a relative risk of 9.2 (ref. 11). We are currently planning to implement multi-stage scoring.

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R. G. CARPENTER

*London School of Hygiene and Tropical Medicine,
Keppel Street,
London WC1*

J. L. EMERY

*The Children's Hospital,
Western Bank,
Sheffield, UK*

Received 16 May; accepted 7 July 1977.

- 1 Carpenter, R. G. & Emery, J. L. *Nature* 250, 729 (1974).
- 2 Carpenter, R. G. & Emery, J. L. *SIDS* 1974 (ed. Robinson, R. R.) 91–96 (Canadian Foundation for the Study of Infant Deaths, Toronto and London, 1974).
- 3 Hughes, E. *Practitioner* 192, 534 (1964).
- 4 Alberman, E. D. & Goldstein, H. *Br. J. prev. soc. Med.* 24, 129 (1970).
- 5 Knox, E. G. & Mahon, D. F. *Archs Dis. Childh.* 45, 634 (1970).
- 6 Emery, J. L. & Carpenter, R. G. *SIDS* 1974 (ed. Robinson, R. R.) 97–106 (Canadian Foundation for the Study of Infant Deaths, Toronto and London, 1974).
- 7 Froggatt, P., Lynas, M. A. & MacKenzie, G. *Br. J. prev. soc. Med.* 25, 119 (1971).
- 8 Emery, J. L. *J. clin. Path.* 26, 386 (1973).
- 9 Smith, B. A. M. *Br. med. J.* iv, 741 (1974).
- 10 Magura, S. *Nature* 256, 519 (1975).
- 11 Carpenter, R. G., Gardner, A. McWeeny, P. M. & Emery, J. L. *Archs Dis. Childh.* (in the press).

Hypolimnas bolina (L.), a mimic of danaid butterflies, and its model *Euploea core* (Cram.) store cardioactive substances

Hypolimnas bolina is a sexually dimorphic nymphalid butterfly with a west to east distribution from Madagascar to Easter Island, and north to south from Japan to Australasia. To the west the female is monomorphic, mimicking species of the oriental and Australasian danaid genus *Euploea*¹. Eastwards *H. bolina* is frequently polymorphic and most forms are then non-mimetic. In areas where it resembles *Euploea* the butterfly has usually been designated a Batesian mimic. The supposed model feeds on Asclepiadaceae and Moraceae only, two families of plants of which various species possess emetic, toxic and purgative properties, whereas the foodplants of *Hypolimnas* include examples from eleven families, only a few of which are irritant or toxic². We now report that when the larva of *H. bolina* feeds on *Ipomoea batatas*, the butterfly stores cardio-active substances. Dried specimens of this butterfly, reared on *Asystasia gangetica*, a plant which does not contain these substances (Table 1), evoke no reaction on isolated rat heart, but living specimens produce a slight response. This indicates that in addition to material sequestered from foodplants, the butterfly itself secretes some type of cardioactive substance. A loss of intrinsic toxic qualities after death has been demonstrated in almost all aposematic model Lepidoptera³, whereas stored secondary plant substances can usually be recorded from an analysis of dried material.

Possibly relevant to our findings with *H. bolina* is the

fact that this butterfly has several features unusual in classical Batesian mimics: (1) the mimetic pattern has not broken down in Madagascar where the model is lacking; (2) although polymorphic—like many Batesian mimics—several of the forms are non-*Euploea*-like and have no recognised model butterflies flying with them; (3) *H. bolina* is remarkably long-lived, a characteristic usually associated with toxic models rather than innocuous mimics; (4) inheritance of the wing pattern is different from that found in the mimetic papilios⁴. In *H. bolina* there is no genetic linkage between the locus controlling the mimicry and that responsible for the other polymorphic forms⁵.

We have investigated the toxicity or pharmacological activity of *H. bolina* and its model *Euploea core*, extracts of the butterflies and their foodplants being tested on the isolated rat and chicken heart (Langendorff preparation⁶) and fed by gavage to pigeons. The insects were bred as described before¹ and their origins are shown in Table 1.

Our results are set out in Tables 1 and 2 and Fig. 1. As Table 1 shows, chicken heart responded to extracts of the model *E. core*, reared either on *Nerium oleander* or three *Asclepias* species, by a reduction in both heart rate and strength of contraction. This suggests that *E. core* stores cardenolides, presumably sequestered from the foodplant², although other cardioactive substances may also be present, masked by plant material. But no emetic effects were produced by feeding pigeons with extracts of either fresh or dried adults or pupae (several trials), showing that the butterfly is not such an effective storer of cardiac glycosides as another model, *Danaus plexippus* (ref. 6 and C. A. Dixon, J. M. Erickson, M.R. and D.N.K., in preparation). The situation is reminiscent of that recorded for the African model of the related *Hypolimnas misippus*, *Danaus chrysippus*^{7,8}, in which sequestration and storage is less effective

Table 1 Effects of adult butterfly extracts on isolated heart

	Foodplant	Sex	No. of experiments	Av. no. of insects extracted per experiment	Av. wt of insects extracted per dose (mg)	Heart tested	% Change in heart rate (mean and range)	% Change in strength of contraction
<i>Euploea core</i> asela Sri Lanka	<i>Nerium oleander</i>	Female	6	3.1	86	Chicken	-32.8 (-20, -60)	-26.5 (-11, -66)
	<i>Asclepias curassavica</i>	Male	4	2.8	101	Chicken	-16.8 (0, -65)	-13.8 (0, -48)
	<i>Gomphocarpus fruticosus</i>	Female	1	3	59	Chicken	-19	-11
<i>Euploea core</i> amymone Hong Kong	<i>Nerium oleander</i>	Male	1	3	84	Rat	-28	+20
	<i>Asclepias curassavica</i>	Female	1	4	73	Chicken	-26	-26
	<i>Gomphocarpus fruticosus</i>	Male	1	3	58	Rat	-37	+32
	<i>Asclepias curassavica</i>	Male	1	3	191	Rat*	-29	+23
	<i>Gomphocarpus fruticosus</i>	Female	1	2	60	Chicken	-15	-15
	<i>Calotropis procera</i>	Female	2	3	126	Rat†	-30 (-30)	+21 (+16, +26)
<i>Hypolimnas bolina</i> Sri Lanka, Fiji, Sabah	<i>Ipomoea batatas</i>	Male	1	3	191	Rat	-29	+23
	<i>Asystasia gangetica</i>	Female	1	2	59	Chicken	-21	-16
	<i>Ipomoea batatas</i>	Male	2	3	139	Rat	-24.5 (-19, -30)	+18 (+16, +20)
	<i>Asystasia gangetica</i>	Female	3	5	89	Rat‡	-42 (-5, -71)	+51 (+20, +66)
	(dried material)	Female	1	5	33	Rat	0	0
	(dried material)	Male	1	7	44	Rat	0	0
	(fresh material)	Female	1	5	121	Rat	Trace of cardiac activity	—
	(fresh material)	Male	1	5	58	Rat	Trace of cardiac activity	—

Euploea extracts were made from live butterflies, *Hypolimnas* extracts from dried material (storage time 2–3 yr) and live material. Extracts from *Hypolimnas* females fed on *Ipomoea*, when injected into the haemocoel of locusts, caused paralysis after 10 min followed by death after 24 h. Neither extracts of dried *Hypolimnas* nor extracts of fresh or dried *Euploea* caused emesis after administration to the pigeon by gavage. Heart rate and strength of contraction were measured for 30 s beginning 10 s after the injection of extract. The control injection effect due to the saline diluent used in mixing extracts is seen in all three figures as a drop in strength almost immediately after injection. Figure 1a shows the less common injection artefact of raising the baseline.

* See Fig. 1b.

† See Fig. 1a.

‡ See Fig. 1c.

and more variable, depending not only on the type of food-plant but on other, probably genetic, factors.

There seemed to be no consistent differences between the cardioactivity produced by the two subspecies of the model *E. core asela* and *E. core amymone*, or by either sex, when fed on the same plants.

Hypolimnas bolina (Table 1), when reared on *Ipomoea batatas* (Convolvulaceae), contained cardioactive substances which also reduced heart rate and increased strength of contraction of the rat heart. Similar activity was produced by extracts of the foodplant *Ipomoea* (Table 2), which genus contains an unusually high proportion of aposematic herbivores⁹.

Although the concept of mimicry is now firmly established there is still discussion about the limitations of Batesian and Müllerian categories. It has recently been recognised that the role of model and mimic can vary according to local conditions and geographical distribution^{7,8,10}. Thus a model can be more or less toxic or distasteful according to the secondary plant substances available in its diet.

Our experiments with captive and wild birds (ref. 1 and

unpublished results of M.R.) have shown that *H. bolina* is not a distasteful species for Corvidae, passerines and quails (*Coturnix*), but that *E. core*, in spite of its lack of emetic qualities, is rejected by these captive birds (unpublished results of M.R.). The presence of cardioactive substances in *H. bolina* when it is reared on *I. batatas* indicates that this butterfly is also an opportunistic sequesterer of active secondary plant substances, and can probably acquire other deterrents such as pyrrolizidine alkaloids from *Senecio* (a recorded foodplant) should the opportunity arise. The foliage of *I. batatas* has been suspected of poisoning stock in Arizona and Australia^{11,12} and there is a report of a severe outbreak of gastro-enteritis in a Bombay jail traced to this source¹³.

In addition to specific glycoses with purgative activity, *Ipomoea* has been shown to contain indole derivatives (containing the nucleus of lysergic acid) and dendrolasin¹⁴, a sesquiterpene. Any of these toxic substances might be sequestered by *H. bolina* from a particular strain of *I. batatas*.

Hypolimnas is a genus which contains about 30 species, two-thirds of which are mimics of danaiids. From our

Fig. 1 On each figure the horizontal line indicates 10 s; extracts were given at the points shown by the arrows. Fluctuations in heart rate and strength of contraction in the first 6–10 s after administration of the extract are injection artefacts and have been ignored in the calculations of results shown in Table 1. *a*, Effect of *Euploea core amymone* (*Gomphocarpus fruticosus*-fed females) extract on the rate and strength of contraction of the isolated rat heart (each vertical trace represents one heart beat). *b*, Effect of *Euploea core amymone* (*A. curassavica*-fed males) extract on the isolated rat heart. *c*, Effect of *Hypolimnas bolina* (*Ipomoea*-fed females) extract on the isolated rat heart.

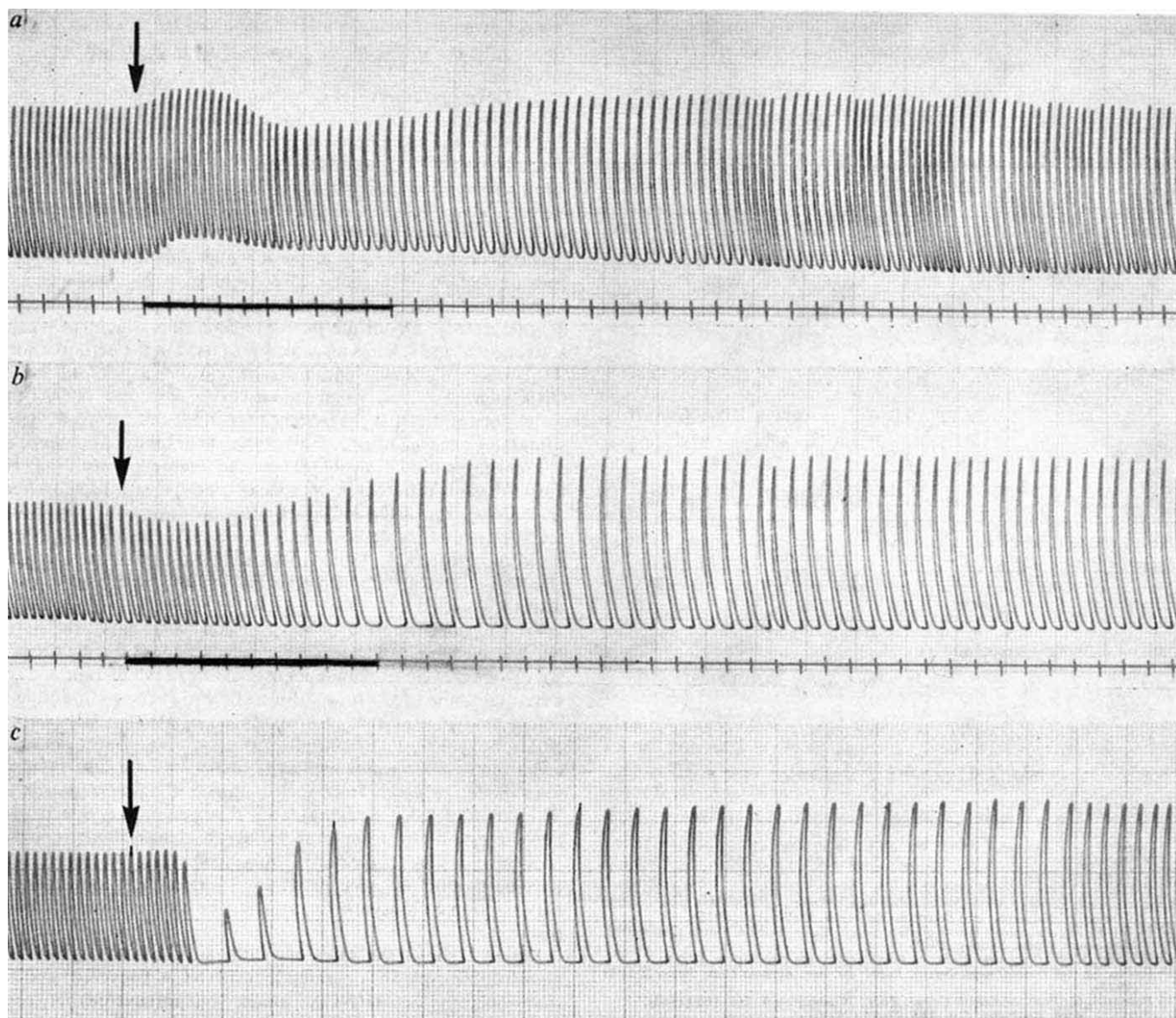


Table 2 Effects of foodplant extracts on the isolated heart and on the pigeon

	No. of experiments	Av. wt of plant extracted per dose (mg)	Heart tested	% Change in heart rate (mean and range)	% Change strength of contraction	Effect of feeding extract of foodplant by gavage*
<i>Ipomoea batatas</i>	3	111	Rat	-47 (-3, -100)	+36 (0, +86)	0
<i>Asystasia gangetica</i>	1	58	Chicken	-15	-8	
<i>Nerium oleander</i>	1	60	Rat	0	0	ND
<i>Asclepias curassavica</i>	1	60	Chicken	0	0	
<i>Gomphocarpus fruticosus</i>	1	114	Rat	-16	-41	+++
<i>Calotropis procera</i>	1	157	Chicken	+100	+72	
	2	115	Rat	-58.5 (-52, -65)	-10 (-5, -15)	+
	1	160	Chicken	-33	+36	
				ND	ND	+++
				ND	ND	++

Extracts were of foliage, except for *Ipomoea* for which both stems and foliage were extracted. Extracts of *Ipomoea* had no effect on the locust after injection into the haemocoel.

*ND, not done; +++, vomiting within 5 min; ++, within 6-14 min; +, within 15-30 min.

preliminary experiments we draw the tentative conclusion that *H. bolina* has a special type of Müllerian mimicry relationship with *Euploea*, and according to circumstances and the species of foodplant available, either species can play the part of model or mimic and have co-equal 'common warning coloration'. Others¹⁵⁻¹⁷ have drawn attention to the unusual distribution of *H. misippus* and its model, and it seems likely that this species, which at times also feeds on *Ipomoea* (unpublished results of David Smith) demonstrates a similar type of relationship to that of *H. bolina* and *E. core*.

N. A. MARSH

Department of Physiology,
Queen Elizabeth College,
Campden Hill Road,
London W8, UK

C. A. CLARKE

Department of Genetics,
University of Liverpool,
Liverpool, UK

MIRIAM ROTHSCILD

Ashton, Peterborough, UK

D. N. KELLETT

Huntingdon Research Centre,
Huntingdon, UK

Decay in juvenile hormone biosynthesis by insect corpus allatum after nerve transection

THE ability of the insect corpus allatum (CA) to synthesise and release juvenile hormone (JH) is believed to be controlled by various neural and humoral influences of both stimulatory and inhibitory nature¹. In last instar *Galleria mellonella*, a CA stimulatory neurohormone (allatotropin) originating in the medial neurosecretory cells of the brain and operating during the early part of the instar has been proposed^{2,4}. In the later part of the instar the synthesis and/or release of JH is inhibited by nervous impulses originating in the brain and reaching the CA through nervous connections². A similar nervous inhibition of JH synthesis is thought to operate in adult *Pyrrhocoris apterus*⁵ and in the cockroaches *Leucophaea maderae*⁶ and *Diploptera punctata*⁷, because section of the nervi corporis allati 1 (NCA 1) seems to activate the CA. A neurohormonal factor from the medial neurosecretory cells of the pars intercerebralis which causes the activation of the CA has also been proposed in the orthopterans, *Anacridium aegyptium* and *Locusta migratoria*⁸. In still other Orthoptera, *Schistocerca* sp., the necessity for intact NCA 1 for activation of the CA has been suggested, indicating a direct stimulatory neural influence rather than an inhibitory influence or a humoral stimulation^{9,10}. Thus the factors controlling JH biosynthesis in different insect species are not clear. We show here that disruption of the NCA 1 by section during the first gonotrophic cycle of the desert locust *Schistocerca gregaria* results in a rapid and predictable decline in the ability of the CA to synthesise JH. The effect of section of the NCA 1 on JH biosynthesis is detectable within 3 h.

To determine the effect of nerve section on JH biosynthesis, the NCA 1 to each CA was severed at selected times during the first gonotrophic cycle of female locusts. After nerve section, locusts were maintained for up to 4 d in standard rearing conditions¹¹ before assaying the rate of biosynthesis of JH ($C_{16}JH$ —methyl 10,11-epoxy-3, 7, 11-trimethyl-*trans*, *trans*-2, 6-dodecadienoate) using our *in vitro* radiochemical procedures^{12,13}. Observations were made on glands from locusts during the first gonotrophic cycle (8-13 d after fledging) and changes in the rate of JH biosynthesis of up to several hundredfold have been reported previously during this period¹¹. Maximal rates of JH biosynthesis have been observed to occur on day 11 (ref. 11) and the effect of nerve section on biosynthetic rates may be most easily observed at this time.

Figure 1 shows the rates of synthesis of JH during the first gonotrophic cycle for operated locusts (NCA 1 severed) and for sham-operated locusts (NCA 1 touched but not severed) between 1 and 4 d after the operation. The lines join the median values for

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- Clarke, C. A. & Sheppard, P. M. *Phil. Trans. R. Soc. B* **272**, 229-265 (1975).
- Common, I. F. M. & Waterhouse, D. F. *Butterflies of Australia* (Angus & Robertson, Sydney, 1972).
- Marsh, N. & Rothschild, M. *J. Zool., Lond.* **174**, 89-122 (1974).
- Langendorff, O. *Arch. ges. Physiol.* **65**, 355-400 (1897).
- Reichstein, T., von Euw, J., Parsons, J. A. & Rothschild, M. *Science* **161**, 861-866 (1968).
- Roeske, C. N., Seiber, J. N., Brower, L. P. & Moffitt, C. M. in *Recent Advances in Phytochemistry* 10 (eds Wallace, J. W. & Mansell, R. L.) 93-167 (Plenum, London & New York, 1975).
- Rothschild, M., von Euw, J., Reichstein, T., Smith, D. A. S. & Pierre, J. *Proc. R. Soc. B* **190**, 1-31 (1975).
- Brower, L. P., Edmunds, M. & Moffitt, C. M. *J. Ent.* **A49**, 183-196 (1975).
- Rothschild, M. in *Insect/Plant Relationships* (ed. van Emden, H. F.) 59-83 (Blackwell, Oxford, 1972).
- Bullini, L. & Sbordoni, V. *Boll. Zool.* **38**, 502 (1971).
- Webb, L. J. *Guide to Medicinal and Poisonous Plants of Queensland* Bull. no. 232 (Council of Industrial and Scientific Research, Melbourne, 1943).
- Hurst, E. *The Poisonous Plants of New South Wales* (Poison Plants Committee, New South Wales, Sydney, 1942).
- Kirkhar, K. R. *Poisonous Plants of Bombay* (Private circulation, 1896).
- Pavan, M. *Gli Iridoidi negli Insetti. Pubbl. Ist. ent. Agraria Univ. Pavia* **2**, 1-49 (1975).
- Bernardi, G. *Proc. Sixteenth Congr. Zool., Washington* **4**, 161-166 (1963).
- Pierre, J. *Arch. Zool. exp. Gen.* **114**, 73-96 (1973).
- Smith, D. A. S. *Biol. J. Linn. Soc.* **8**, 183-204 (1976).

each day. Differences between median values for the various times after nerve section are small on any given day (on days 9 and 11), and cannot be distinguished in Fig. 1. At all times the median JH biosynthetic rate in sham-operated animals was greater than that in operated animals. The median values for operated animals were consistently low throughout the experiment and no differences were apparent between animals in which the NCA 1 were severed for various times. The sham-operated values reached a maximum on day 11, in good agreement with results for unoperated normal animals¹¹. These results indicate that within 1 d of section of the NCA 1, the CA lost the ability to biosynthesise JH, and did not regain it for at least 4 d.

Because of this rapid loss in CA activity, the JH biosynthetic abilities of CA were determined at 3–6 h intervals during the first 24 h after the operation. Locusts 10–11 d after fledging were used because of the high JH biosynthetic rates observed at this time. Figure 2 shows that CA activity again decreased rapidly after nerve section; within 3 h values for operated animals were consistently lower than those for sham-operated animals, and by 6 h, JH biosynthesis by CA of operated animals was low. The assay procedure included a 3 h incubation of CA *in vitro* so that the values in Fig. 2 represent the rates of JH biosynthesis during a 3 h interval after the elapsed post-operative period. Thus the values at 3 h, for example, actually represent the rate of JH biosynthesis between 3 and 6 h after the initial operation; similarly, 12 h values represent the rate of biosynthesis between 12 and 15 h after nerve section.

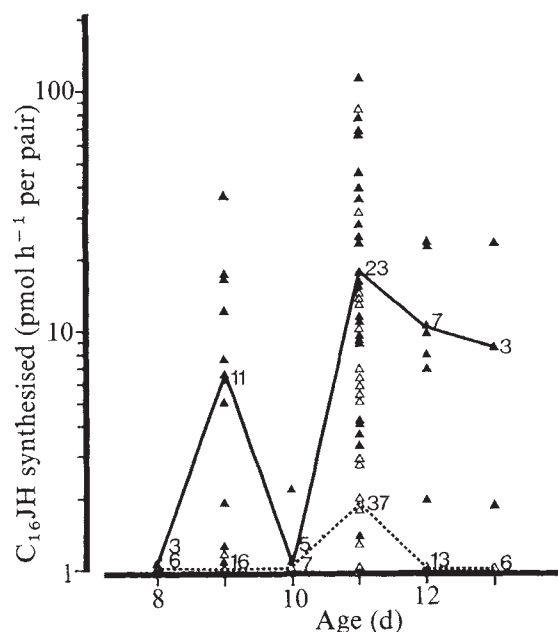


Fig. 1 The rate of synthesis of C_{16} JH by corpora allata during the first gonotrophic cycle of *Schistocerca gregaria* at various times after section of the NCA 1. Values on day 8, 2 d after nerve section; day 9–2 and 3 d after section; day 10, 3 d after section; day 11, 1 and 4 d after section; day 12, 2 d after section; day 13, 3 d after section. Each point represents a single determination on an individual pair of glands. Δ , Animals in which the NCA 1 was sectioned; $\blacktriangle$, sham-operated animals. The dotted line joins the median values of NCA 1-sectioned animals and the solid line joins the median values of sham-operated animals. The numbers adjacent to the median values represent the total number of determinations on any given day. In every case, the median rates of synthesis in operated animals are lower than the sham-operated animals and never greater than 2 pmol per h per pair. The rates of synthesis of C_{16} JH were monitored by incubation of the glands for 3 h in TC 199 (GIBCO, 25 mM HEPES) containing Ficoll (20 mg ml⁻¹) and methyl ¹⁴C-methionine (Amersham-Searle; final specific radioactivity 35–37 mCi mmol⁻¹). After incubation, glands plus medium were extracted with 15 volumes of $CHCl_3$: C_2H_5OH (5:1) after adjustment to pH 4.6 and addition of appropriate non-radioactive marker compounds. Extracts were analysed by thin layer chromatography (Merck F₂₅₄ silica gel) in ethyl acetate:n-hexane (25:75). Radioactivity in C_{16} JH was quantified by liquid scintillation spectrometry.

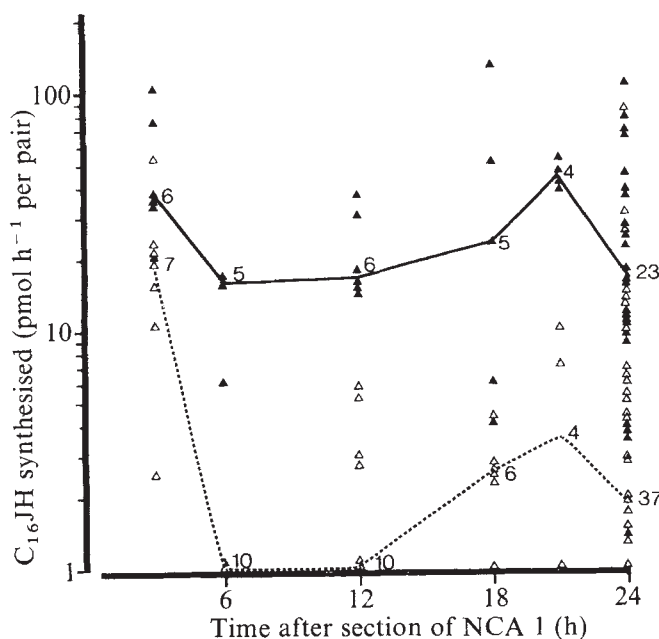


Fig. 2 The rate of synthesis of C_{16} JH by corpora allata on day 11 after fledging at various times after section of the NCA 1. Each point represents a single determination on an individual pair of glands. Symbols and methods as for Fig. 1. In every case, the median rates of synthesis in operated animals are lower than those of the sham-operated animals. The effect of nerve section can be seen within 3 h of the operation and by 6 h, the rate of synthesis has dropped to a low level. Section of the NCA 1 results in a rapid decline in C_{16} JH biosynthesis.

It is useful to compare the decay in CA activity after nerve section with the time course of release of JH by locust CA *in vitro* (completely denervated). It has been shown that JH is released at a constant rate from isolated CA of *S. gregaria* for 3 h *in vitro*¹⁴ and release then declines rapidly, principally in CA with high synthetic activity. Our results *in vivo* confirm these observations because there was a significant decrease in the rate of JH biosynthesis within 6 h of nerve section, that is, 3 h after nerve section *in vivo* + 3 h incubation *in vitro*. CA with low rates of JH release (low activity CA), however, continue to release JH at a constant rate for at least 5 h (ref. 14). These CA *in vitro* have rates of biosynthesis similar to both those CA with high synthetic activity after 3 h *in vitro* and those CA observed *in vitro* after nerve section. This suggests that CA lacking the appropriate 'neural' input synthesise JH at a low but constant rate for some time, whether *in vivo* or *in vitro*. Section of the NCA 1 in animals with active CA therefore resulted in a rapid decline in CA activity whereas section of the NCA 1 in animals with CA of low synthetic activity did not significantly alter the rate of JH biosynthesis. There was a large between-animal variation in the rates of biosynthesis in both unoperated locusts¹¹ and in sham-operated animals (Figs 1 and 2) and we attribute much of this to the difficulty in obtaining completely synchronised populations of animals, when the activity of the CA is changing so rapidly¹¹. Because not all of the experimental CA exhibit high activity at any given time, the effect of nerve section on CA activity is probably more pronounced than Figs 1 and 2 indicate.

It may be suggested that section of the NCA 1 damages the CA, thus altering their ability to synthesise JH. To test this possibility, we incubated CA from both operated and sham-operated animals at two times after nerve section, in the presence of the exogenous precursor farnesenic acid. Farnesenic acid has been demonstrated previously to undergo rapid conversion to C_{16} JH through esterification and epoxidation^{12,15}, and any damage to the cells of the CA presumably would be reflected in an impaired ability to effect these terminal critical steps in JH biosynthesis. Table 1 shows the biosynthetic rates by CA from 11-d locusts 12 and 24 h after section of the NCA 1, incubated in the presence of farnesenic acid. *t* tests on these values at each time revealed that there was no

Table 1 C_{16} JH synthesis *in vitro* in presence of farnesenic acid after section of the NCA 1

Adult age (d)	Time after section of NCA 1 (h)	C_{16} JH Synthesis (pmol per h per pair)	
		Operated	Sham-operated
11	12	Mean 167.9 ± 20.4 Median 171.5 ($n=10$)	181.1 ± 23.2 183.1 ($n=8$)
11	24	Mean 95.1 ± 7.62 Median 95.1 ($n=7$)	121.3 ± 13.0 118.0 ($n=7$)

Mean values are $\pm$ s.e.m.; n , no. of animals assayed. There is no significant difference between operated and sham-operated values. At 12 h, $P > 0.6$ and at 24 h, $P > 0.05$.

significant difference in JH biosynthesis between operated and sham-operated CA ($P > 0.6$ at 12 h; $P > 0.05$ at 24 h). This demonstrates that section of the NCA 1 does not alter the ability of the CA to effect the final two stages in JH biosynthesis. In addition, these results suggest that the stimulatory or sustaining factor(s) arriving at the CA through the NCA 1 do not exert their effects by altering the activities of the two terminal enzymic stages. It has been suggested that rate limitation in JH biosynthesis does not occur at the final two stages in *S. gregaria*¹⁵, and our results support this suggestion.

Our results show that the presence of intact NCA 1 is necessary for the continued biosynthesis of JH by active CA in this species and provide a possible explanation for the difficulties encountered by some authors when attempting to promote sexual maturation or the expression of juvenile characters through the implantation of homologous CA (refs 16–18). The CA from adults of certain other species seem to respond differently. For example, CA from female *Periplaneta americana* take more than 36 h to reduce their rate of incorporation of 14 C-methionine into C_{16} JH when isolated in chemically-defined culture medium²⁰, and there is physiological evidence that these glands may retain significant levels of synthetic activity for several weeks when transplanted to larval *Rhodnius prolixus*²¹. These other CA may be less acutely dependent upon the integrity of their nervous connection to the brain than those of *S. gregaria*, and in some cases may be able to respond independently to trophic humoral factors if these are present.

Our results suggest that if a stimulatory or sustaining factor regulates JH biosynthesis in adult *S. gregaria*, this factor does not normally operate by a humoral pathway. Massive electrical stimulation of the pars intercerebralis of *L. migratoria* and *A. aegyptium* has been reported to result in activation of denervated inactive CA (ref. 8). We suggest that although massive release of neurosecretory material may result in activation of the CA by a humoral pathway in experimental conditions, this is not the normal pathway in *Schistocerca*. Rather the factor must reach the CA by the nervous tracts. Other authors have shown that electrical stimulation of the brain in *S. gregaria* also results in activation of intact CA (ref. 19). Thus activation may occur by conventional nervous pathways as well. It is not known if the 'allatotropic' factor is the product of neurosecretory cells or conventional neurones or if both types of products are involved in the activation of the CA. With regard to the latter possibility, conventional neurones may regulate the release of neurosecretory products within the CA. Such a mechanism may regulate the release of egg-laying hormone (a neurosecretion) in *Aplysia*^{22,23}.

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S. S. TOBE
C. S. CHAPMAN

Department of Zoology,
University of Toronto,
25 Harbord Street,
Toronto, Ontario,
Canada M5S 1A1

G. E. PRATT

Unit of Invertebrate Chemistry and Physiology,
Agricultural Research Council,
University of Sussex,
Falmer, Brighton, UK

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- Engelmann, F. *The Physiology of Insect Reproduction* (Pergamon, Oxford, 1970).
- Granger, N. A. & Sehna, F. *Nature* **251**, 415–417 (1974).
- Sehna, F. & Granger, N. A. *Biol. Bull.* **148**, 106–116 (1975).
- Granger, N. A. & Borg, T. K. *Gen. comp. Endocr.* **29**, 349–359 (1976).
- Hodkova, M. *Nature* **263**, 521–523 (1976).
- Scharrer, B. *Biol. Bull.* **102**, 261–272 (1952).
- Engelmann, F. *Biol. Bull.* **116**, 406–419 (1959).
- Moulins, M., Girardie, A. & Girardie, J. *Nature* **250**, 339–340 (1974).
- Strong, L. J. *Insect. Physiol.* **11**, 271–280 (1965).
- Pener, M. P. *J. Zool.* **147**, 119–136 (1965).
- Tobe, S. S. & Pratt, G. E. *J. exp. Biol.* **62**, 611–627 (1975).
- Pratt, G. E. & Tobe, S. S. *Life Sci.* **14**, 575–586 (1974).
- Tobe, S. S. & Pratt, G. E. *Biochem. J.* **144**, 107–113 (1974).
- Pratt, G. E., Tobe, S. S., Weaver, R. J. & Finney, J. R. *Gen. comp. Endocr.* **26**, 478–484 (1975).
- Pratt, G. E., Tobe, S. S. & Weaver, R. J. *Experientia* **31**, 120–122 (1975).
- Highnam, K. C., Lusi, O. & Hill, L. J. *Insect. Physiol.* **9**, 587–596 (1963).
- Pener, M. P. *J. Insect Physiol.* **13**, 665–684 (1967).
- Staal, G. B. *Publ. Fonds Lundb. Export Bur.* 1916–1918 **40**, 1–125 (1961).
- Highnam, K. C. *J. Microsc.* **103**, 57–72 (1962).
- Pratt, G. E., Weaver, R. J. & Hammett, A. F. in *The Juvenile Hormones* (ed. Gilbert, L. I.) 164–178 (Plenum, New York, 1976).
- Wigglesworth, V. B. *J. exp. Biol.* **29**, 620–631 (1952).
- Kupferman, I. & Kandel, E. R. *J. Neurophysiol.* **33**, 865–876 (1970).
- Pinkser, H. M. & Dudek, F. E. *Science* **197**, 490–493 (1977).

Sex hormone of *Dictyostelium discoideum* is volatile

THE macrocyst pathway of development represents the sexual cycle of the cellular slime moulds^{1–4}. O'Day and Lewis⁵ have shown that the sexual interaction between opposite mating types of *Dictyostelium discoideum* is mediated by a low molecular weight, diffusible hormone. They also showed that only one mating-type strain (NC-4) was a secretor of the sexual hormone while the opposite strain (V-12) was a responder (that is, formed macrocysts). This sexual interaction was verified by MacHac and Bonner⁶ and was later shown to exist in another species, *D. purpureum*⁷. In our attempts to characterise the sexual system of *D. discoideum*, our results have revealed that the sexual hormone of this species is volatile.

Figure 1 depicts the construction of the diffusion chamber and outlines the culture methods used in this study. The stock cultures of the mating-type strains (NC-4 and V-12)

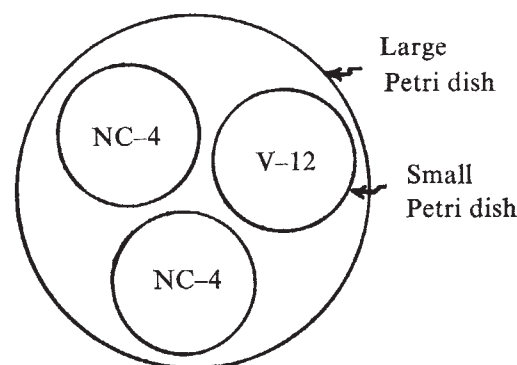


Fig. 1 Diagram of the diffusion chamber with an example of the most successful inducing situation. Small Falcon plastic Petri dishes (30 × 10 mm) containing 4.0 ml 0.1% L-P agar (0.1% Difco peptone, 0.1% Difco lactose, 1.5% Difco agar) were inoculated with 0.5–1.0 ml suspension of *E. coli* B/r and spores (1–2.5 × 10⁴ spores) of one mating-type strain of *D. discoideum*. Three of these small dishes were placed uncovered in a large Fisher plastic Petri dish (100 × 15 mm) which was then covered and sealed with tape (masking tape or 3M vinyl electrical tape). The sealed plates were placed in light-tight metal canisters and stored for two weeks at 23 ± 1 °C before observation.

Table 1 Macrocyt formation in diffusion chamber cultures

Experimental situation	No. of chambers set up	No. of small plates with pre-cyst clumps*		No. of small plates with mature macrocysts*	
		V-12	NC-4	V-12	NC-4
Two NC-4; one V-12	42	30/42	0/84	6/42	0/84
Two V-12; one NC-4	20	5/40	0/20	0/40	0/20
Two d-H ₂ O; one V-12	10	0/10	NA	0/10	NA
Three NC-4	20	NA	0/60	NA	0/60
Three V-12	20	0/60	NA	0/60	NA

*Macrocyt formation involves an initial aggregation of cells to form discrete, tight cell clumps (pre-cyst clumps) which, in appropriate conditions, form dormant structures possessing three cell walls (mature macrocyt). Data are expressed as ratios of the total number of small plates with pre-cyst clumps or mature macrocysts to total number of small plates used for that strain.

used to inoculate the small Petri dishes were maintained on *Escherichia coli* strain B/r on 0.1% L-P agar at 22–24 °C in the presence of light⁸. The three small plates of the diffusion chamber were inoculated in the following combinations: two NC-4 and one V-12; two V-12 and one NC-4; two distilled water and one V-12; three NC-4; three V-12. After two weeks the small plates of each chamber were examined for the presence of macrocysts.

The results of these experiments are summarised in Table 1. Pre-cyst cell clumps and mature macrocysts were only observed in cultures of V-12. Furthermore, these structures were only observed when at least one NC-4 culture was present in the diffusion chamber, whereas mature macrocysts were only observed when two NC-4 cultures were present. In other experiments, seven of the small Petri dishes (six NC-4; one V-12) were placed in a very large, sealed, plastic Petri dish (150×20 mm) in an attempt to increase the level of macrocyt formation. Only a low level of macrocyt formation by V-12, however, was observed, possibly due to the large air space in such containers. The fact that only V-12 cultures contained macrocysts argues that cell migration from one small dish to another did not occur. As an additional control, however, in several experiments a 'moat' of sterile, distilled water was placed around the base of the small Petri dish containing the V-12 culture. After the two-week period, the contents of the moat were examined by phase contrast microscopy. Amoebae were never observed in this fluid, re-emphasising that cellular migration between plates did not occur.

In a different but related series of experiments, ten mixed mating-type cultures of NC-4 and V-12 were left unsealed, whereas ten were sealed. In these experiments, the sealed dishes routinely showed 500 or more macrocysts per plate, whereas the unsealed cultures had 50–100 or fewer.

The results thus reveal that the sexual hormone of *D. discoideum* is volatile. This organism, however, is not unique, since volatile sex hormones are produced by many other eukaryotic microbes⁹. In addition, volatile molecules may play a role in other aspects of slime mould development such as in the spacing of fruiting bodies, in the formation of microcysts and in homothallism^{10,11}. The data are also consistent with the concept that macrocyt formation is at least a two-step phenomenon. Since pre-cyst clumps only formed in cultures which had a ratio of one NC-4 to two V-12, whereas mature macrocysts were formed by V-12 when two NC-4 cultures were present, it is likely that a low level of hormone may induce pre-cyst formation, while a higher level of this hormone or, possibly, a second volatile molecule may be required for events to proceed past this point.

Finally, the fact that the aggregation-inducing factor for sexual interaction is volatile at 23±1 °C, whereas cyclic AMP is not, reveals that the events of cell aggregation in these two alternative developmental events are different. Presently we are continuing our attempts to identify the sexual hormone of *D. discoideum*.

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KEITH E. LEWIS
DANTON H. O'DAY

Department of Zoology and Erindale College,
University of Toronto,
Mississauga, Ontario,
Canada L5L 1C6

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- Clark, M. A., Francis, D. & Eisenberg, R. *Biochem. biophys. Res. Commun.* **52**, 672–678 (1973).
- Erdoş, G. W., Raper, K. B. & Vogen, L. K. *Proc. natn. Acad. Sci. U.S.A.* **70**, 1828–1830 (1973).
- Erdoş, G. W., Raper, K. B. & Vogen, L. K. *Proc. natn. Acad. Sci. U.S.A.* **72**, 970–973 (1975).
- Macinnes, M. A. & Francis, D. *Nature* **251**, 321–323 (1974).
- O'Day, D. H. & Lewis, K. E. *Nature* **254**, 431–432 (1975).
- MacHac, M. A. & Bonner, J. T. *J. Bact.* **124**, 1624–1625 (1975).
- Lewis, K. E. & O'Day, D. H. *Can. J. Microbiol.* **22**, 1269–1273 (1976).
- Nickerson, A. W. & Raper, K. B. *Am. J. Bot.* **60**, 190–197 (1973).
- Gooday, G. W. *Rev. Biochem.* **43**, 35–50 (1974).
- Lonski, J. *Dev. Biol.* **51**, 158–165 (1976).
- Weinkauff, A. M. & Filosa, M. F. *Can. J. Microbiol.* **11**, 385–387 (1965).

Modification of circadian flight activity in the mosquito *Anopheles gambiae* after insemination

THE flight activity of female *Anopheles gambiae* Giles, recorded in the laboratory, follows a bimodal cycle¹. In LD12:12 (alternating 12 h light:12 h dark) the first peak comes shortly after light-off and there is a secondary phase of activity which reaches its maximum in the middle or latter part of the dark phase. This secondary activity occurs at the time when host seeking and biting take place in the wild²; the initial peak corresponds with the time of egress of mosquitoes from their daytime resting places and, in particular, in virgin females, with the time of mating³. In normal conditions, *A. gambiae* females are inseminated only once in their lifetime^{4,5}, and usually not before they are 2–3 d old (ref. 3). We show here that, after insemination, the first peak of activity is greatly reduced and the secondary phase of activity is enhanced. This switch in activity is likely to represent a change from mating to host seeking behaviour.

Pala strain female mosquitoes, were sugar-fed and reared at 26 °C in an LD12:12 regime. Males and females were separated following emergence. When 3–4 d old, half the females were placed in a cage for 24 h with twice the number of males; the insemination rate was approximately 90%. The flight activity of similar numbers of individual virgin and mated females was then recorded for a week, using the acoustic actograph technique^{6,7}. The experiments were first carried out in an LD12:12 regime with a sharp change from light to dark and vice versa, and then repeated using an electronically produced artificial dawn and dusk⁸ with the transition lasting approximately 75 min. After each experiment, survivors were dissected and spermathecae examined.

Figure 1 shows the mean flight activity of virgin and inseminated females 6 d after emergence, that is, in the case of the

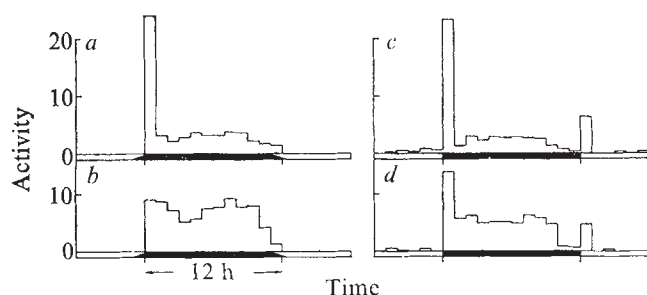


Fig. 1 Flight activity (mean score per h) of sugar-fed *A. gambiae* females in LD12:12. *a, b*, With an artificial dawn and dusk: *a*, virgins ($n = 29$) and *b*, inseminated ($n = 28$). *c, d*, With sharp light-on and light-off: *c*, virgins ($n = 42$) and *d*, inseminated ($n = 25$).

mated females, 24 h after the end of their confinement with the males. In both types of regime, inseminated females show a significant decrease in amount of activity in the first hour of the dark period (t test, $P < 0.01$ in both cases) and an increase in the secondary activity (t test, $P < 0.02$ in both cases). The net effect is an increase of 30–50% in the total activity during the dark phase. The same pattern was observed throughout the experiment. The difference in activity between virgin and inseminated females is more immediately apparent in dawn and dusk conditions. Possibly the peak shown by the inseminated females in Fig. 1*d* is partly a response to the sharp change at light-off; the peak at light-on has already been shown to be a response to such a sharp change¹.

Craig and his co-workers^{8,9} have demonstrated that in *Aedes aegypti* and a number of other mosquitoes, the female is rendered refractory to further insemination through the action of the male accessory gland secretion, which is transferred to the female in the seminal fluid. These secretions may also affect the female by stimulating oviposition, modifying biting behaviour, increasing the rate of digestion of blood meals and possibly enhancing longevity^{10,11}. It seems probable that the behavioural changes we have observed are a consequence of the transference of accessory gland substance. The switch in behaviour could be particularly important in a species such as *A. gambiae* in which mating and feeding may take place at entirely different sites.

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M. D. R. JONES
S. J. GUBBINS

School of Biological Sciences,
University of Sussex,
Brighton, UK

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- ¹ Jones, M. D. R., Cubbin, C. M. & Marsh, D. *J. exp. Biol.* **57**, 347–357 (1972).
- ² Gillies, M. T. & DeMeillon, B. *The Anophelinae of Africa South of the Sahara*. *Pub. S. Afr. Inst. med. Res.* **54** (1968).
- ³ Charlwood, J. D. thesis, Univ. Sussex (1976).
- ⁴ Goma, L. K. H. *Nature* **197**, 99–100 (1963).
- ⁵ Bryan, J. H. *Nature* **218**, 489 (1968).
- ⁶ Jones, M. D. R., Hill, M. & Hope, A. M. *J. exp. Biol.* **47**, 503–511 (1967).
- ⁷ Jones, M. D. R., Cubbin, C. M. & Marsh, D. *J. exp. Biol.* **57**, 337–346 (1972).
- ⁸ Craig, G. B., Jr. *Science* **156**, 1499–1501 (1967).
- ⁹ Gwadz, R. W., Craig, G. B., Jr & Hickey, W. A. *Biol. Bull.* **140**, 201–214 (1971).
- ¹⁰ Ramalingham, S. & Craig, G. B., Jr *Canada Entomol.* **108**, 955–960 (1976).
- ¹¹ Downe, A. E. R. *J. Insect. Physiol.* **21**, 1835–1839 (1975).

2,4-D plasmids and persistence

ACCORDING to the UN Food and Agricultural Organisation production year book of 1974, 27×10^6 kg of the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) were consumed in 1973, making this the most extensively used of all pesticides. One desirable characteristic of 2,4-D, apart from its efficacy as a herbicide, is its lack of persistence in the environment¹,

due to degradation by a variety of microorganisms. We have argued that in view of the isolation of plasmids encoding the degradation of naturally occurring aliphatic and aromatic compounds², there exists a class of plasmids which specifically encode the degradation of many man-made derivatives of these compounds, in this instance 2,4-D. Using segregation and transfer analyses we have demonstrated that a plasmid or plasmids is involved in 2,4-D degradation. Clearly bacteria have evolved which can degrade many different pesticides; our results indicate that plasmids may have an important role in this evolution.

Initial attempts to study 2,4-D degrading ability in soil bacteria were hampered by the marked instability of this character in many of our isolates. We have isolated a strain of *Pseudomonas* (JMP116) that degrades 2,4-D and retains this characteristic even after subculture in the absence of 2,4-D. Degradation of 2,4-D by JMP116 was detected by acid production in Loos' media³, disappearance of the 283-nm absorption peak⁴ and growth on 2,4-D as a sole source of carbon and energy. Since JMP116 does not degrade phenoxycetic acid, we have concluded that this pathway is specific for 2,4-D.

In view of the segregation of 2,4-D negative clones by certain of our isolates we examined the possibility that these genes are plasmid borne. We have found that the wild-type JMP116 is readily cured of its 2,4-D degrading ability of mitomycin C. After JMP116 at 10^7 colony-forming units (CFU) per ml was inoculated into PYE broth containing $0.1 \mu\text{g ml}^{-1}$ mitomycin C and grown at 32°C with aeration for 18 h, 40–60% of the clones re-isolated on PYE were cured of 2,4-D degrading ability. Control cultures without mitomycin C show $< 1\%$ spontaneous loss of this character. Since mitomycin C can act as a mutagen, especially in strains which are sensitive to the antibiotic, we tested one cured isolate (JMP119) for back mutation to the wild type, 2,4-D degrading phenotype. This cured strain does not revert to wild type at a detectable level.

Segregation and curing are characteristics of plasmid genes⁵. For many plasmids it is possible to isolate plasmid-specific bacteriophage⁶. PR11 is a plasmid-specific bacteriophage that formed plaques on strains harbouring RP1, a *pseudomonas* R plasmid with an exceptionally wide host range⁷. This bacteriophage also plaques on JMP116, but not on JMP119 (a JMP116 derivative cured of its ability to degrade 2,4-D). It is likely that JMP116 harbours a conjugative plasmid related to RP1 that encodes 2,4-D degradation. This has been confirmed by an intrastain mating between JMP116 and a nalidixic acid resistant mutant of JMP119. The results (Table 1) show that the plasmid is transferred with a frequency of 5×10^{-2} per donor cell in an overnight mating, and there is a 100% correlation between the acquisition of the plasmid and the ability to degrade 2,4-D. Although failure to separate the conjugative phenotype from the 2,4-D degrading phenotype in either curing (unpublished data) or transfer experiments suggests that the 2,4-D degrading ability is encoded by a conjugative plasmid, it must be recognised that more than one plasmid may be involved—for instance a non-conjugative plasmid, specifying the degradative pathway, being mobilised by a conjugative plasmid. Information on the actual number of plasmid species involved in 2,4-D degradation is being sought by the isolation and characterisation of plasmid DNAs from JMP116 and JMP119.

Torstenonson *et al.*⁸ have reported that the continued application of 2,4-D results in a progressive decrease in its persistence. They point out that the adaption hypothesis does not explain why this increased ability to degrade 2,4-D persists from one year to the next. Our results show that the presence of pesticide degrading plasmids have an important role in the lack of persistence of 2,4-D. This report of a pesticide degrading plasmid and its relationship

Table 1 The relationship between the inheritance of a conjugative plasmid and the ability to degrade 2,4-D

Possible phenotypes	No. of colonies
<i>Ped</i> ⁺ ϕ^r	0
<i>Ped</i> ⁺ ϕ^s	52
<i>Ped</i> ⁻ ϕ^r	1,000
<i>Ped</i> ⁻ ϕ^s	0

Ped^{+/−}, ability/inability to degrade 2,4-D. $\phi^{r/s}$, Resistance/sensitivity to the plasmid specific bacteriophage PR11. Intrastrain mating between JMP116 (*Ped*⁺) and a nalidixic acid-resistant mutant of JMP119 (*Nal*^r *Ped*[−]). Exponential (about 1×10^9 CFU ml^{−1}) cultures of the donor (JMP116) and the recipient (JMP119 *Nal*^r) were diluted one-tenth in PYE broth, mixed in equal volumes and incubated without aeration at 32 °C overnight (18 h). The recipient was re-isolated on PYE agar containing 20 μ g ml^{−1} nalidixic acid. The re-isolated recipient clones were tested for their susceptibility to the plasmid-specific bacteriophage PR11 and ability to degrade 2,4-D in Loos' media³.

to RPI raises a number of important questions. How widespread are pesticide degrading plasmids and to what extent do they contribute to the degradation of the myriad of man-made pesticides in use today? Are pesticide-degrading plasmids analogous to antibiotic-resistant plasmids in that the application of the pesticides elicits an epidemic spread of the plasmid through the soil microbial population?

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J. M. PEMBERTON
P. R. FISHER

Department of Microbiology,
University of Queensland,
St Lucia, Queensland,
Australia

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¹ Alexander, M. *Soil Biology. Reviews of Research*, Natural Resources Research, Unesco 9, 209–240 (1969).

² Chakrabarty, A. M. *A. Rev. Genet.* 10, 7–30 (1976).

³ Loos, M. A. *Can. J. Microbiol.* 21, 104–107 (1975).

⁴ Burger, K., MacRae, I. C. & Alexander, M. *Proc. Am. Soil Soc.* 26, 243–246 (1962).

⁵ Lacy, R. W. *Bact. Rev.* 39, 1–32 (1975).

⁶ Stanisich, V. A. *J. gen. Microbiol.* 84, 332–342 (1974).

⁷ Haas, D. & Holloway, B. W. *Molec. gen. Genet.* 144, 243–251 (1976).

⁸ Torstensson, N. T. L., Stark, J. & Goransson, B. *Weed Res.* 15, 159–164 (1975).

An antiviral substance extracted from *Streptococcus faecalis*

ALTHOUGH there are a number of reports on virus-inhibiting substances, knowledge of those of bacterial origin is rather limited^{1–6}. Horsfall and co-workers^{7,8} reported that polysaccharides of *Klebsiella pneumoniae* suppressed multiplication of pneumonia virus of mouse *in vivo* and of mumps *in ovo*. Carver *et al.*^{9–11} described virus-inhibiting factor(s) which they isolated from different species of bacteria belonging to both Gram-positive and Gram-negative groups. We report here that a substance or substances extracted from *Streptococcus faecalis* inhibited proliferation of certain human pathogenic viruses *in vitro* and *in vivo*.

S. faecalis organisms cultivated in VF bouillon supplemented with 1% glucose and 0.04% cystine were washed and sonicated (20 KHz, 30 min). Supernatant fluid obtained by a centrifugation (12,000g, 30 min) was the crude extract; the protein content was determined by the method of Lowry *et al.*¹². Test virus was usually herpes simplex virus type 1 (HSV-1) propagated in HeLa-S₃ cell cultures, and in addition adenovirus type 12 (Ad-12) grown in KB cells.

The HSV suspension and the extract were simultaneously introduced into HeLa monolayer cultures in bottles and incubated at 37 °C for 1 h to allow complete cell-virus adsorption. After removing the excess fluid, overlay medium consisting of methylcellulose (0.5%), calf serum (3%) and kanamycin (60 μ g ml^{−1}) in Eagle's minimum essential medium was added. Following incubation at 37 °C for 4 d, the cultures were stained with 1% crystal violet in ethanol. Numbers of plaques thus formed were significantly reduced than those in control cultures added with 0.01 M phosphate-buffered saline (PBS, pH 7.0) (ref. 13) in place of the bacterial extract.

The crude extract was subjected to salting out by ammonium sulphate and to acetone treatment, then gel filtration with Sephadex G-100. Particular fractions were comparatively effective in the plaque-inhibiting capacity; typical examples are shown in Fig. 1.

The antiviral substance (AVS) also exhibited an *in vivo* activity. AVS and HSV-1 were simultaneously injected into mice intraperitoneally (i.p.) and mortality ratios of AVS-treated mice were significantly lower than those of control mice given virus alone (Table 1).

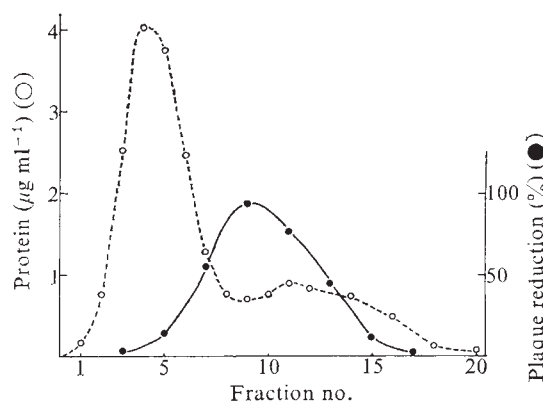


Fig. 1 Profile of Sephadex G-100 gel filtration of an ammonium sulphate precipitate of *Streptococcus faecalis* extract. The bacterial extract was prepared by the procedures indicated in text and adjusted to contain 15 mg ml^{−1} protein by addition of 0.01 M PBS (pH 7.0). Ammonium sulphate was then added to 70% saturation and stirred for 30 min at 5 °C. After a centrifugation at 15,000g for 30 min, the supernatant was brought to 90% saturation of (NH₄)₂SO₄ and stirred for 30 min at 5 °C. The precipitate collected by centrifugation (15,000g, 30 min) was dissolved in PBS and dialysed against PBS for 24 h at 5 °C. The non-dialytic fluid was adjusted to contain 8 mg ml^{−1} protein with PBS and gently added with acetone to 40% concentration, which was placed at −10 °C for 20 min. Following a centrifugation (10,000g, 30 min), acetone was added to the supernatant to a final concentration of 45% and again placed at −10 °C for 20 min. The precipitate collected by centrifugation (10,000g, 30 min) was then dissolved in PBS and dialysed against PBS at 5 °C for 24 h. After the non-dialytic fluid was adjusted to contain 40 mg ml^{−1} protein with PBS, the solution (5 ml) was charged on a Sephadex G-100 column (26 × 700 mm, Pharmacia, previously equilibrated with PBS) and eluted with PBS. Fractions (6 ml each) were collected and each of them was adjusted to contain 800 μ g ml^{−1} protein. One-tenth ml each of the samples and 0.1 ml of HSV-1 suspension (adjusted to contain 100 PFU) were simultaneously introduced into HeLa cell monolayer cultures to determine plaque titres.

Similar activity was seen in experiments using suckling hamsters infected with Ad-12 virus. Development of virus-induced tumours was suppressed by administration of AVS. The effect was unequivocal when the AVS was given simultaneously with the virus, but the same substance given 15 d after virus inoculation was much less active (Table 2).

AVS (300–1,000 μ g protein per ml) had no direct effect on the infectivity of viruses suspended in culture medium or PBS, and showed no acute toxicity to HeLa cells (at least as determined by morphology under an ordinary light microscope and proliferation capacity in bottles). Injection

of AVS i.p. into 3-week-old white mice (320 µg as protein per mouse once a day for 10 days) brought about no loss of body weight, compared with control untreated mice. The antiviral activity resisted heating at 95 °C for 5 min or treatment with RNase or lipase, while it was completely lost or significantly reduced by treatment with proteolytic enzymes such as pronase or trypsin. The AVS was non-dialytic. These data suggest that the active principle is a protein.

AVS apparently lacked activity as an interferon stimulus. Mice, aged 3 weeks, were injected i.p. with AVS (60–1,500 µg protein in 0.4 ml PBS, per mouse). Plasma was taken after 3, 6 and 24 h and interferon titres were determined by the method of Younger *et al.*¹⁴ using vesicular stomatitis virus (VSV) and mouse L cells. No interferon induction was noted in any of the test systems. We also found that the VSV-susceptibility of L cells pretreated with AVS was practically the same as that of control untreated cells; no difference of VSV titres was noted between these two cellular systems.

Table 1 Curative effect of AVS on HSV-1 infection in mice

Group of mice	Mortality ratio of mice		
	Experiment I	Experiment II	Experiment III*
AVS-treated	5/30 (17)†	5/23 (22)	14/85 (16)
Control	24/30 (80)	19/24 (79)	66/85 (78)

Three-week-old male mice (ddY-F strain) were each injected with HSV-1 i.p. (2,000 plaque-forming units each) and AVS (320 µg protein) simultaneously. Mice in control groups received the same virus alone. Observation periods ended at 14 d after the injection. Differences of mortality ratios between the AVS-administered and control groups are significant at the confidence level of 95%.

*Summary of 11 separate experiments carried out in the same way.

†Numerator indicates number of mice which died showing typical signs of HSV-1 infection, and denominator indicates number of mice inoculated; figure in parenthesis indicates percentage.

Some of the mechanism(s) underlying the phenomena were investigated as follows. The HeLa cell monolayer cultures were given HSV-1 and AVS simultaneously. After being incubated at 37 °C for 1 h, the viral titres of fluid phase were determined. As shown in Fig. 2, the amounts of virus which remained unadsorbed in the fluid of AVS-administered cultures were significantly higher than those of the same virus present in the fluid of control cultures receiving PBS in place of the AVS. It was also noted that the amounts of unadsorbed virus were almost constant regardless of the titres of original virus inocula, both in the AVS-administered and control cultures. It seemed that the AVS affected the virus at the stage of adsorption on to host cells.

Extracts were prepared from other kinds of bacteria and tested for virus-inhibiting capacity as above. In all 19

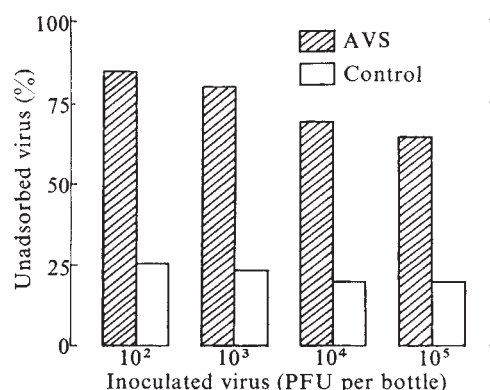


Fig. 2 Amounts of HSV-1 remaining unadsorbed in the fluid phase of HeLa-S₃ monolayer cultures receiving simultaneously virus plus AVS or virus plus PBS. HSV-1 (titres as indicated in abscissa) and AVS (160 µg protein) were simultaneously introduced on to HeLa monolayer cultures in bottles. Control cultures received the same virus plus PBS instead of the AVS. After incubation at 37 °C for 1 h, excess fluid was taken and assayed for the plaque titres which were taken to indicate amounts of virus unadsorbed. PFU, plaque-forming units.

strains of organism of 17 species, including *S. lactis*, *Lactobacillus acidophilus*, *L. casei*, *Bifidobacterium bifidum*, *Escherichia coli* and *Proteus vulgaris* were examined. None of these samples was superior to the AVS from *S. faecalis* in the antiviral effects. It therefore seems that the virus-inhibiting activity described here may be species-specific.

SUSUMU HOTTA

MASAMI KOJIMA

MASAHITO FUJISAKI

SHINSUKE UCHIDA

Department of Microbiology,
Kobe University School of Medicine,
Kobe, Japan

HISATORA KURODA

Department of Microbiology,
Kobe Women's College of Pharmacy,
Kobe, Japan

CHUYA HAMADA

Department of Virology,
Niigata University School of Medicine,
Niigata, Japan

Table 2 Inhibitory effect of AVS on development of tumours induced by Ad-12

Inoculum*	Tumour incidence (%)	P§
Experiment I		
Ad-12† plus AVS‡ simultaneously	9/19 (47.3)	< 0.01
Ad-12 plus AVS 15 days later	17/20 (85.0)	> 0.2
Ad-12 alone	19/20 (95.0)	
Experiment II		
Ad-12 plus AVS simultaneously	4/11 (36.4)	< 0.05
Ad-12 plus AVS 15 days later	10/11 (90.9)	> 0.2
Ad-12 alone	11/12 (91.7)	

*Inoculated subcutaneously in the back of 1-day-old hamsters.

† 5×10^6 TCID₅₀ (tissue culture infecting dose) in 0.05 ml; Huie strain grown in KB cells.

‡0.265 mg protein in 0.05 ml.

§Calculated by the χ^2 test.

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- Tamm, I. & Eggers, H. J. in *Viral and Rickettsial Infections of Man* 4th edn (eds Horsfall, F. L., Jr & Tamm, I.) 305–338 (Lippincott, Philadelphia, 1965).
- Herrmann, E. C., Jr *et al.* *Ann. N.Y. Acad. Sci.* 130, 1–482 (1965).
- Second Conference on Antiviral Substances* (eds Herrmann, E. C., Jr & Stinebring, W. R.) *Ann. N.Y. Acad. Sci.* 173, 1–844 (1970).
- Antivirals with Clinical Potential* (ed. Merigan, T. C.) (University of Chicago Press, Chicago, 1976).
- Becker, Y. *Antiviral Drugs, Monographs in Virology*, XI (ed. Melnick, J. L.) (Karger, Basel, 1976).
- Third Conference on Antiviral Substances* (ed. Herrmann, E. C., Jr) *Ann. N.Y. Acad. Sci.* 284, 1–720 (1977).
- Horsfall, F. L., Jr & McCarty, M. J. *exp. Med.* 85, 623–646 (1947).
- Ginsberg, H. S., Goebel, W. F. & Horsfall, F. L., Jr *J. exp. Med.* 87, 385–410 (1948).
- Carver, D. H. & Naficy, K. *Proc. Soc. exp. Biol. Med.* 111, 356–360 (1962).
- Carver, D. H. & Naficy, K. *Proc. Soc. exp. Biol. Med.* 116, 548–552 (1964).
- Carver, D. H. & Rosen, F. S. *Proc. Soc. exp. Biol. Med.* 116, 575–579 (1964).
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. *J. biol. Chem.* 193, 265–275 (1951).
- Dulbecco, R. & Vogt, M. J. *exp. Med.* 99, 167–182 (1954).
- Younger, J. S., Keleti, G. & Feingold, D. S. *Inf. Immun.* 10, 1202–1206 (1974).

An immunological basis for inhibition of transformation of human lymphocytes by EB virus

It is well known that Epstein-Barr virus (EBV) transforms human lymphocytes *in vitro*, and lymphoblastoid cell lines are established¹. Macrophages enhance transformation by EBV *in vitro*² but may not be essential³. Inhibition of transformation by EBV *in vitro* has also been reported, and was first observed when lymphocytes from an adult donor with antibody to EBV were infected with EBV and co-cultivated with autochthonous fibroblasts derived from skin⁴. Fibroblasts of foetal origin did not have this inhibitory effect. Further studies established the principle that co-cultivation of EBV-infected lymphocytes with autochthonous or allogeneic fibroblasts of adult but not foetal origin, was associated with inhibition of transformation by EBV (ref. 5). This report presents evidence that this type of inhibition of transformation by EBV is specifically mediated by mononuclear cell preparations from donors previously infected with EBV. An immunological basis is highly probable.

The major characteristics of the experimental system for studying inhibition of transformation by EBV are noted briefly. First, inhibition is defined as the failure of non-adherent lymphocytes to aggregate and proliferate yet still remain infected and viable. Second, inhibition has not been shown to be due to a factor released by adult fibroblasts. Third, inactivation of the inoculated EBV is not the cause of inhibition because an EBV-associated nuclear antigen (EBNA) is demonstrable in the inhibited lymphocytes for several weeks⁵. Fourth, the inhibition is reversible up to at least four weeks after inoculation of EBV by simply adding phytohaemagglutinin to the cultures⁶ or by removing the non-adherent lymphocytes from the adult fibroblasts and transferring them to a clean tissue culture vessel^{5,6}. Finally, reversal of inhibition results in proliferation of the EBV-infected cells within a few days, and typical lymphoblastoid cell lines emerge⁵. The proliferation of cell lines already transformed by EBV is not inhibited in the presence of adult fibroblasts⁵.

In a series of experiments, inhibition of transformation by EBV was consistently recorded when EBV-infected lymphocytes of three donors with antibody to EBV were co-cultivated with fibroblasts from skin of two adult donors, but not when they were co-cultivated with fibroblasts derived from four different foetuses. In contrast, inhibition of transformation by EBV was not demonstrated with lymphocytes from a donor without antibody to EBV. This observation prompted a detailed investigation of the relationship between inhibition of transformation by EBV *in vitro* and the previous history of EBV infection of the lymphocyte donor.

The methods used in this work have been described in detail^{4,7}. In essence, mononuclear cell populations were prepared from peripheral blood and the adherent cells removed by incubation in medium containing autochthonous serum in plastic Petri dishes. The non-adherent mononuclear cell population (mainly lymphocytes) was infected with filtered EBV of the QIMR-WIL strain and cultured in microtitre plates. Appropriate fibroblast cultures were usually prepared on the previous day. The cultures (at least six replicates) were maintained in medium RPMI 1640 containing 16% inactivated foetal calf serum and observed microscopically for evidence of transformation for at least 4 weeks, although transformation was usually evident in 7 d.

The results of investigations on 14 donors (10 males and 4 females, aged 5–46 yr) were quite clearcut. Non-adherent lymphocytes from each donor, whether with or without antibody to EBV, were uniformly transformed in the usual manner by EBV when cultivated alone or with foetal fibroblasts. A strikingly different pattern occurred in the presence of adult fibroblasts; transformation occurred uniformly with lymphocytes from all eight donors (six adults and two children) without

antibody to EBV but with none of the lymphocyte samples from six donors (five adults and one child) with antibody to EBV. In addition, lymphocytes from four umbilical cord blood samples transformed as usual on adult fibroblasts.

In the work reported here, the lymphocyte concentration was 0.5×10^6 ml⁻¹ and the multiplicity of EBV infection (MOI) 0.1 TD₅₀ per cell. Inhibition was also observed with a MOI of 15 and a cell concentration ranging from 10^5 – 10^6 ml⁻¹, showing that the phenomenon was not dependent on the dose of virus or the cell concentration. Two experiments were carried out in a different manner. Non-adherent lymphocytes from donors without previous EBV infection (adult and cord) were infected with EBV, mixed with uninfected lymphocytes from donors with previous EBV infection, and co-cultivated with adult fibroblasts. In these conditions, viral transformation was inhibited as described above, confirming that inhibition of transformation is dependent on the presence of lymphocytes from EBV-immune donors.

The effect of previous EBV infection on the role of adult fibroblasts in the inhibition of EBV transformation was also studied. The results, summarised in Table 1, showed that inhibition was dependent on previous EBV infection of the donor of the lymphocytes but not of the donor of the adult fibroblasts.

From the present results it now seems likely that an immunological system capable of inhibiting transformation of lymphocytes by EBV is inherent in the mononuclear cells from persons with previous EBV infection. Detection of this inhibition is so far dependent on particular culture conditions, and the role of fibroblasts of adult origin seems to be simply to provide these conditions. Failure of non-adherent mononuclear cells to inhibit transformation when cultivated alone or in the presence of fibroblasts of foetal origin may be due to unsuitable culture conditions or to interference by other cellular factors. The mechanism of inhibition is unknown but it may well involve a complex interaction of various immunological components. These include the expression of EBV antigens on infected target cells, the particular EBV-specific immunological effector system involved, the survival and activity of the components of the effector system in various culture conditions, and the influence of various mitogenic factors. Obvious possibilities are that the inhibition involves T lymphocytes, antibody, or antibody-mediated cellular immunity, acting at the surface of the EBV-infected cells to modulate their behaviour. These aspects are being examined.

Table 1 Effect of previous EBV infection on role of fibroblasts during inhibition of EBV transformation

Leukocyte donor and reciprocal antibody titre to viral capsid antigen	Fibroblast donor and reciprocal antibody titre to viral capsid antigen	Proportion of cultures transformed
P.G.P. (<10)	S.G.B. (160)	6/6
	B.D.B. (<10)	6/6
	Foetal (NT)*	6/6
	None	6/6
	S.G.B. (160)	6/6
K.D. (<10)	B.D.B. (<10)	6/6
	Foetal (NT)	6/6
	None	6/6
	S.G.B. (160)	0/6
	B.D.B. (<10)	0/6
S.G.B. (160)	Foetal (NT)	6/6
	None	6/6
	S.G.B. (160)	0/12
	B.D.B. (<10)	0/12
	Foetal (NT)	12/12
D.J.M. (40)	None	12/12
	S.G.B. (160)	12/12
	B.D.B. (<10)	12/12
	Foetal (NT)	12/12
	None	12/12
Umbilical cord (NT)	S.G.B. (160)	12/12
	B.D.B. (<10)	12/12
	Foetal (NT)	12/12

*NT, Not tested.

Inhibition of transformation by EBV without death of the target cells contrasts with the cytotoxicity for EBV-infected target cells of selected lymphocyte populations from patients with acute infectious mononucleosis⁸⁻⁹. Lymphocytes from persons with longstanding EBV infections were not cytotoxic in those two systems. The specificity of inhibition of transformation contrasts also with the nonspecific inhibition by human lymphocytes of EBV lymphoblastoid cell lines¹⁰⁻¹², providing further support for the view that inhibition of EBV transformation acts at a point before the process is complete.

The manner in which EBV persists following infectious mononucleosis is of considerable interest. It has been suggested that cells transformed by EBV persist in the body probably under immunological control¹³, or that the EBV genome may persist in cells in an incompletely expressed form¹⁴. The present studies of inhibition of transformation bear on this particular problem as well as on wider questions of viral latency, especially with herpesviruses.

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D. J. MOSS
W. SCOTT
J. H. POPE

Queensland Institute of Medical Research,
Brisbane, Australia

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- ¹ Henle, W., Diehl, V., Kohn, G., zur Hausen, H. & Henle, G. *Science* **157**, 1064-1065 (1967).
- ² Pope, J. H., Scott, W. & Moss, D. J. *Nature new Biol.* **246**, 140-141 (1973).
- ³ Schneider, U. & zur Hausen, H. *Int. J. Cancer* **15**, 59-66 (1975).
- ⁴ Pope, J. H., Scott, W. & Moss, D. J. *Int. J. Cancer* **14**, 122-129 (1974).
- ⁵ Moss, D. J., Pope, J. H. & Scott, W. *Med. Microbiol. Immun.* **162**, 159-167 (1976).
- ⁶ Pope, J. H. in *Oncogenesis and Herpesviruses II, IARC Scientific Publications No. 11*, Part 1 (ed. de-Thé, G., Epstein, M. A. & zur Hausen, H.) 367-378 (IARC, Lyon, 1975).
- ⁷ Moss, D. J. & Pope, J. H. *Int. J. Cancer* **15**, 503-511 (1975).
- ⁸ Svedmyr, E. & Jondal, M. *Proc. natn. Acad. Sci. U.S.A.* **72**, 1622-1626 (1975).
- ⁹ Rickinson, A. B., Crawford, D. & Epstein, M. A. *Clin. exp. Immun.* **28**, 72-79 (1977).
- ¹⁰ Takasugi, M., Mickey, M. R. & Terasaki, P. I. *Cancer Res.* **33**, 2898-2902 (1973).
- ¹¹ Hutt, L. M., Huang, Y. T., Dascomb, H. E. & Pagano, J. S. *J. Immun.* **115**, 243-248 (1975).
- ¹² Jondal, M. & Pross, H. *Int. J. Cancer* **15**, 596-605 (1975).
- ¹³ Henle, W. & Henle, G. in *Oncogenesis and Herpesviruses II, IARC Scientific Publications No. 11*, Part 2 (ed. de-Thé, G., Epstein, M. A. & zur Hausen, H.) 215-224 (IARC, Lyon, 1975).
- ¹⁴ Epstein, M. A. in *Oncogenesis and Herpesviruses II, IARC Scientific Publications No. 11*, Part 2 (ed. de-Thé, G., Epstein, M. A. & zur Hausen, H.) 141-151 (IARC, Lyon, 1975).

Intermittent exposure to fluorescent light extends lifespan of human diploid fibroblasts in culture

SHORT exposure (3 or 4 h) to fluorescent light one to three times weekly enhances the proliferation rate of human diploid fibroblasts in culture¹. This proliferative response is reversible and is mediated through the culture medium, Dulbecco Vogt's supplemented with 10% foetal bovine serum. Conceivably, this increase in proliferation rate might also influence the lifespan of human diploid fibroblasts which undergo a limited number of population doublings (PDs) in culture². To evaluate the long term effects of repeated light exposure on lifespan, three lines of human fibroblasts originating from adult skin (HU-274), a mince of whole foetus (HU-278), and foetal lung (WI-38), were assayed in quantitative experiments to determine the lifespan measured as cumulative cell number and total PDs.

Irrespective of the PD level of the cells at the beginning of the experiment, repeated exposure (3 h) to cool white fluorescent light (6 to 9 W m⁻²) three times weekly, reproducibly extended the lifespan. This extension was reflected by an increase in the total number of PDs, cumulative increase in cell number, and duration of cell survival and growth (Figs 1-3). Cessation of growth was indicated by a decrease in cell number from inoculum size after 2 weeks' incubation. The cells of both foetal lines, WI-38 and HU-278, which before this experiment had undergone 44 or

more PDs and had been exposed to room fluorescent lights during routine handling, showed a 19-21% increase in total PDs (Table 1). In contrast, the cells of adult origin, HU-274, which had never been exposed to light of wavelength less than 500 nm during the initial seven PDs in culture showed a 70-98% increase in total PDs. The unexposed cells of line HU-274 underwent a total of 20-25 PDs before cessation of growth. Cells of the exposed lines, on the other hand, have undergone 40-42 PDs to date, and proliferation rates have decreased to a level of 1.5-1.7-fold increase over a 4-week period. Light exposures have been reduced to two per week, and the cultures are being maintained to determine if they can become established as continuous cell lines.

Although the proliferation rate of human fibroblasts in culture may be increased by a number of environmental factors including the volume of culture medium per cell³, serum concentration³, medium formulation (R. Parshad, unpublished), and intermittent light exposure¹, the only factor shown to increase proliferation rate as well as extend the lifespan reproducibly is the addition of cortisone or hydrocortisone to the medium⁴. The addition of hydrocortisone to the medium of WI-38 cells increased the PDs 30-40% above those of untreated controls. The duration of

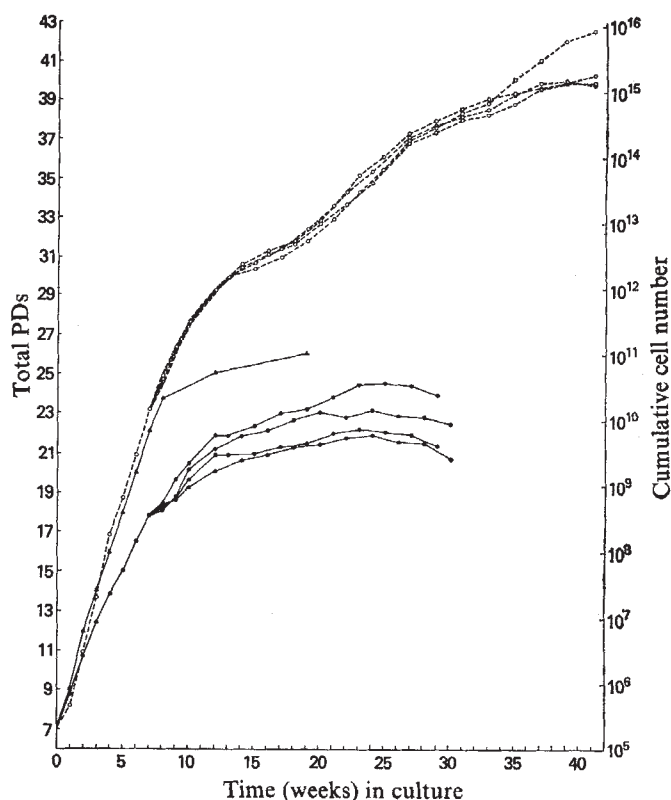


Fig. 1 Effect of intermittent light on lifespan of human skin fibroblasts, line HU-274, derived from a 50-yr-old black male who died of hypertension and renal failure. The cells grown in Dulbecco Vogt's medium supplemented with 10% foetal bovine serum had been carried for seven PDs as estimated from 1:2 or 1:4 split ratios before this experiment. In the quantitative studies, 2×10^5 cells were inoculated into 5 ml medium in a T-25 flask (Falcon). Light-exposed cultures were illuminated under two cool-white fluorescent light tubes (GE F 15T8/CW) at 27 cm from the floor of the flask with an exposure intensity of 6 to 9 W m⁻² for 3 h, three times a week, 24 h after inoculation and twice thereafter following culture fluid renewal. After an interval of 1 or 2 weeks, depending on cell growth, the number of cells per flask was determined by haemocytometer. Cells from replicate cultures were inoculated at 2×10^5 cells per flask to continue the line and quantify as above. The PDs for the shielded, non-quantified cell line are based on 1:2 or 1:4 split ratios. Each point is the average of counts from four replicate cultures. Except for the light exposure described above, cells and culture medium were handled under red or gold light and were never exposed to light of wavelength below 500 nm. ●, Unexposed; ○, light exposed

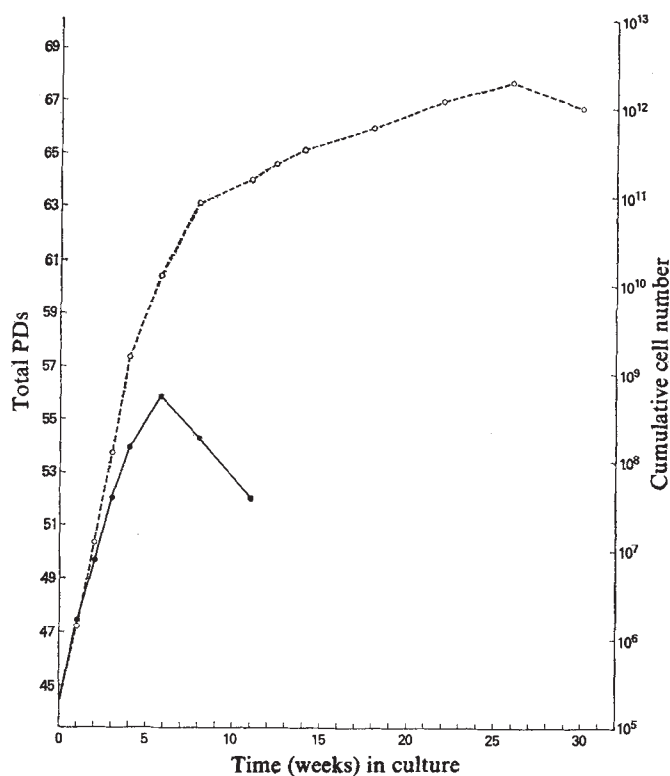


Fig. 2 Effect of intermittent light on lifespan of foetal lung WI-38 cells obtained at passage 17 from the American Type Culture Collection Rockville, Maryland. Before this quantitative experiment, cells had been carried in NCTC 135 with 10% foetal bovine serum for 21 PDs and in Dulbecco Vogt's medium for six PDs based on 1:4 or 1:8 split ratios. For experimental details, see Fig. 1. ●, Unexposed; ○, light exposed.

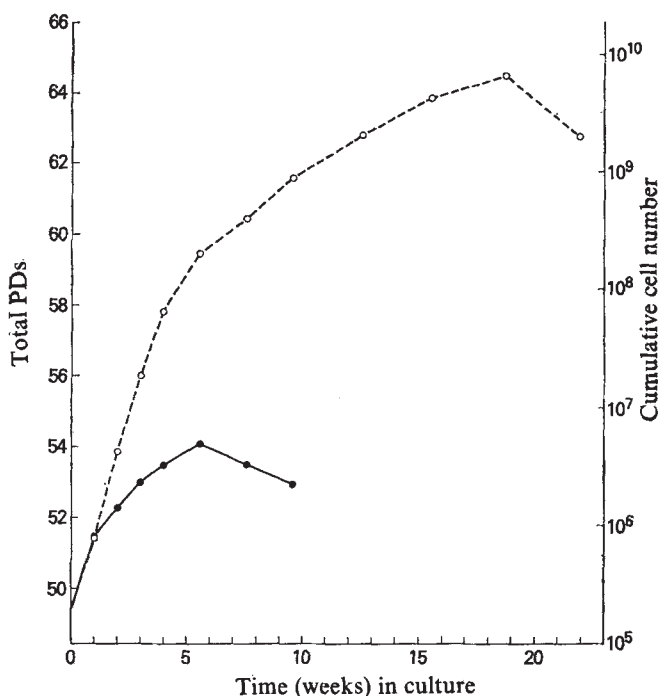


Fig. 3 Effect of intermittent light on lifespan of human foetal cells, line HU-278, derived from a mince of whole foetus. Before this quantitative experiment cells had been carried in NCTC 135 with 10% foetal bovine serum for 38 PDs before cryopreservation, and for 11.5 additional PDs in Dulbecco Vogt's medium based on 1:4 or 1:8 split ratios. For experimental details, see Fig. 1. ●, Unexposed; ○, light exposed.

Table 1 Effect of intermittent light exposure on lifespan (PDs of) human fibroblasts in culture

Cell line	Initial PD before light exposure	Additional PDs*		% Increase in exposed to total unexposed PD†
		Unexposed	Exposed	
HU-274-1	7	17.53	34.79	70.40
-2	7	15.07	32.73	80.02
-3	7	14.55	32.70	84.22
-4	7	12.56	32.76	98.02
HU-278	49.5	4.66	15.04	19.17
WI-38	44.5	11.44	23.25	21.11

*PD (x) was calculated by the equation $N = N_0 2^x$. Where N_0 = initial cell number and N = final cumulative cell number.

†PD of exposed - PD of unexposed ÷ Initial PD + PD of unexposed × 100.

life in culture for both control and treated cells, however, was the same. Intermittent light treatment, here, of WI-38 cells increased the PDs 100% above those of unexposed controls. When intermittent light exposure of adult skin cells (HU-274) was started early in culture, the maximum increase in PDs was 160% above those of unexposed controls. In all three cell lines, the duration of life in culture was also greatly increased.

Although cytotoxic effects of fluorescent light on cells in culture have been reported³, Litwin⁶ observed an increased lifespan but no enhanced proliferation rate in human embryonic fibroblasts when exposed to light for 2 or 4 h daily. In the conditions of the present study, which include a relatively high inoculum size, low light intensity, and short duration of exposure, stimulation of proliferation rate and extension of lifespan were observed in all three cell lines of adult or foetal origin. We have shown previously that the stimulation of proliferation by fluorescent light is mediated through the culture medium. The extension of lifespan may likewise be associated with photochemical changes in some component(s) of the medium.

RAM PARSHAD

Department of Pathology,
College of Medicine,
Howard University,
Washington DC 20059

KATHERINE K. SANFORD

Biochemistry Laboratory,
National Cancer Institute,
National Institutes Health,
US Public Health Service,
Bethesda, Maryland 20014

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¹ Parshad, R. & Sanford, K. K. *J. cell. Physiol.* (in the press).

² Hayflick, L. *Expl Cell Res.* 37, 614-636 (1965).

³ Ryan, J. M., Sharf, B. B. & Cristofalo, V. J. *Expl Cell Res.* 91, 389-392 (1975).

⁴ Macieira-Coelho, A. *Experientia* 22, 390 (1966). Cristofalo, V. J. *Adv. geront. Res.* 4, 45-79 (1972). Cristofalo, V. J. *Adv. exp. Med. Biol.* 61, 57-79 (1975).

⁵ Wang, R. J. *In Vitro* 12, 19-22 (1976). Pereira, O. M., Smith, J. R. & Packer, L. *Photochem. Photobiol.* 24, 237-242 (1976).

⁶ Litwin, J. *Expl Geront.* 7, 381-386 (1972).

Contact-mediated communication of ouabain resistance in mammalian cells in culture

THE active transport of Na^+ and K^+ across the plasma membrane, mediated by Na^+/K^+ -ATPase, can be inhibited specifically by the cardiac glycoside ouabain¹. Ouabain is toxic to mammalian cells in culture, the degree of sensitivity varying with species²; however, cells resistant to its cytotoxic effects have been obtained in rodent³⁻⁴ and human^{5,6} cell strains. Ouabain-resistant (oua^r) cells are thought to have a mutation affecting the Na^+/K^+ -ATPase so that their enzyme has a lower affinity for ouabain than the enzyme of ouabain-sensitive (oua^s) cells. Therefore, oua^r cells can actively

transport Na^+ and K^+ at ouabain concentrations that would inhibit oua^s cells^{2,4,5}. We have observed that oua^s human cells, when cocultured densely with oua^r human cells, acquire a functional resistance to ouabain as evidenced by their ability to proliferate in medium containing the drug (unpublished observations). We suspected that ouabain resistance was communicated from oua^r to oua^s cells and that this transfer was a manifestation of ionic transport through a pathway independent of the $\text{Na}^+/\text{K}^+-\text{ATPase}$. There is substantial evidence that a specialised membrane structure, the gap junction, is the ultrastructural pathway for the transfer of ions, metabolites and other small molecules between cells in contact⁷⁻⁹. To determine whether the acquisition of ouabain resistance we observed was mediated through gap junctions, we have compared—for the ability to communicate ouabain resistance—cells which are known to form gap junctions with those that cannot. Our results indicate that ouabain resistance can be communicated from oua^r to oua^s cells, that the process is contact dependent and that it does not occur in cells unable to form gap junctions.

To assay for communication of ouabain resistance, we analysed chromosome preparations obtained from mixed cultures of oua^r and oua^s cells in ouabain for the presence of metaphase cells of oua^s origin. The ability of oua^s cells to divide in ouabain would provide definitive evidence that the sensitive cells had acquired resistance. We used mouse fibroblasts as the oua^r cells and human fibroblasts as the oua^s cells for the following reasons. Mouse and human metaphases are distinguishable by conventional staining techniques (Fig. 1). Mouse cells are naturally 10^4 -fold more resistant to ouabain than human cells, so they can proliferate in 10^{-6} M ouabain, whereas human cells cannot. There are mouse cell strains that form gap junctions, and others that do not. The oua^r cells were therefore (1) mouse 3T3 fibroblasts, which are known to be communicating because they form gap junctions¹⁰, ionically couple¹¹ and transfer nucleotides¹², and (2) mouse L fibroblasts known to be non-communicating because they do not form gap junctions^{7,8} and do not transfer ions^{7,8} or nucleotides^{7,8,12-14}. The oua^s cells were a strain of human skin fibroblasts (no. 106) isolated in our laboratory from a 2-yr-old female; they were used in their 5-8th subculture and have been shown to communicate nucleotides^{6,14}. Human skin fibroblasts are known to form gap junctions and transfer ions⁸.

Table 1 Percentage human metaphases of total metaphases in cultures of human and mouse cells plated into control and ouabain medium

Experiment	Cells	Ouabain conc. in medium	Total metaphases	% Human metaphases
1	Human	0	200	100
		10^{-6} M	0	0
	Human + L	0	200	45
		10^{-6} M	200	0
	Human + 3T3	0	200	54
2	Human	10^{-6} M	200	51
		0	200	100
	Human + L	0	200	50
		10^{-6} M	200	0
	Human + 3T3	0	200	55
		10^{-6} M	200	49

For experiment 1 cells were plated on to 22-mm² coverslips in 35-mm dishes containing 2 ml of medium and incubated at 37 °C in 5% CO_2 . After 24 h of cultivation, the medium was renewed to ensure stringent selection. After 48 h, coverslips were fixed and stained for chromosome analysis as described in Fig. 1. For experiment 2 cells previously plated on to 22-mm² coverslips were transferred to the same 100-mm dish (Fig. 2) and then treated as in experiment 1. In both experiments cells were plated at the same densities— 10^5 human cells and 5×10^4 mouse cells per 35-mm dish. Cells were maintained in growth medium (Eagle's minimal essential medium supplemented with non-essential amino acids, glutamine, penicillin-streptomycin, fungizone and 20% foetal calf serum (Gibco)). Growth medium containing 10^{-6} M ouabain is referred to as ouabain in the text. Human metaphases were distinguished from mouse at $\times 100$ magnification.

The human cells were plated alone or co-cultivated with either L or 3T3 cells at densities ensuring cell contact^{13,14} in medium containing 10^{-6} M ouabain (hereafter referred to as ouabain). For a control, a similar series was plated into non-selective medium. The cultures were incubated, as described in Table 1, and then chromosome preparations were made. Analysis of the metaphase spreads (Table 1, experiment 1) revealed, as expected, that L and 3T3 cells divided in ouabain, whereas human cells, when plated alone, did not. In the mouse-human cell mixtures plated into ouabain, no human metaphases were found when human cells were co-cultivated with L cells; however, abundant human metaphases were observed when human cells were co-cultivated with 3T3 cells (Fig. 1). Thus, human cells were able to acquire ouabain resistance from 3T3 cells but not from L cells.

To assure that the communication of ouabain resistance observed in the 3T3-human cell mixtures was not attributable to passage of molecules through the medium, the experiment was repeated in the following conditions. Coverslips containing the cell mixtures and appropriate controls were transferred to the same large Petri dish containing either ouabain or non-selective medium (Fig. 2). The results of metaphase analysis from this experiment (Table 1, experiment 2) were identical to those of experiment 1 in which cells had been incubated in individual Petri dishes. The human cells on the same coverslip with 3T3 cells were able to divide in ouabain, whereas human cells in the same dish, but not on the same coverslip with 3T3 cells, were unable to divide. The absence of human metaphases on coverslips containing human cells alone or in combination with L cells not only confirms that L cells cannot transfer ouabain resistance, but also indicates that the communication of ouabain resistance seen with 3T3 cells does not occur through the medium. Moreover, increasing the ratio of 3T3 to human cells (by floating a 22-mm² coverslip containing human cells on a confluent lawn of 3T3 cells in a 100-mm dish) did not enable the human cells to divide in ouabain (data not shown). The best interpretation for our observation is that the communication of ouabain-resistance is dependent on contact between human and 3T3 cells.

Our results demonstrate that L cells, characterised by an inability to form gap junctions, cannot confer ouabain resistance on oua^s cells; whereas 3T3 cells, which are able to form gap junctions, can transfer ouabain resistance. This provides compelling evidence that the gap junction mediates the communication of ouabain resistance and also suggests a molecular basis for this communication. There is substantial evidence from experiments

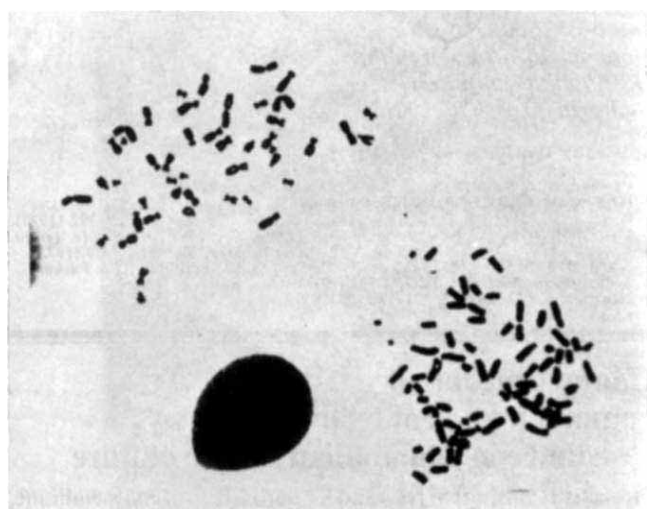


Fig. 1 Photomicrograph showing human and mouse metaphases from mixtures of human and 3T3 cells in 10^{-6} M ouabain. Chromosome preparations were made *in situ*: the cells on 22-mm² coverslips were exposed to colchicine ($0.4 \mu\text{g ml}^{-1}$) for 1 h; 0.075 M KCl for 5 min; and 3:1 methanol:acetic acid for 30 min at 4 °C. Coverslips were then immersed in ice-cold water, dried on a hot plate and stained with Giemsa. A clone of 3T3 cells with very few metacentric chromosomes was used to facilitate discrimination of the metaphases as either mouse or human. ($\times 663$.)

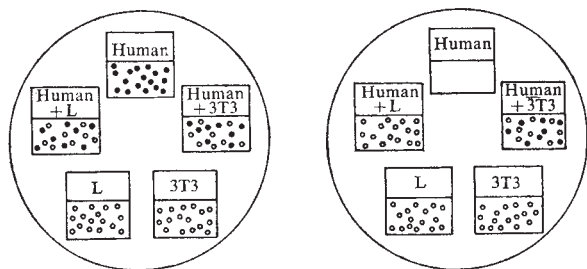


Fig. 2 Experiment demonstrating that communication of ouabain-resistance does not occur through the medium. Mouse and human cells, as indicated, were plated on to 22-mm² coverslips in individual 35-mm dishes containing non-selective medium. After cell attachment (5 h), the coverslips were transferred into a 100-mm dish containing 10 ml of non-selective (a) or ouabain (b) medium and treated as described in Table 1, experiment 2. The results of metaphase analysis are indicated schematically: ●, human metaphases; ○, mouse metaphases. In ouabain only the human cells on the same coverslip with 3T3 cells are able to divide.

involving both ultrastructural and electrophysiological techniques that Na⁺ and K⁺ can diffuse freely from cell to cell through the low-resistance pathways of gap junctions without leakage into the extracellular space^{7-9,11,15-17}. Diffusion of Na⁺ and K⁺ through gap junctions between oua^r and oua^s cells would maintain the correct internal cationic balance in the oua^s cell, even though its own Na⁺-K⁺-ATPase is inhibited by ouabain.

Alternative explanations, for example, transfer of resistant Na⁺-K⁺-ATPases through gap junctions, are unlikely because these junctions are not permeable to molecules greater than 1,200 daltons¹⁸. The large proportion of responding cells precludes a rare event such as spontaneous mutation as a cause; in fact, we have not observed spontaneous mutations to ouabain resistance in 5×10^7 human skin fibroblasts⁶. Nor does the oua^r phenotype significantly affect the ability of a cell to communicate⁶. One cannot exclude the possibility that resistant Na⁺-K⁺-ATPases are transferred to the sensitive cells through focal membrane fusion, or diffusion in the immediate environ of resistant cells, but neither of these nor other mechanisms readily explain the observed differences between 3T3 and L cells. On the basis of our results, the most likely explanation is that resistance is transferred by diffusion of ions through gap junctions.

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CHERYL M. CORSARO

BARBARA R. MIGEON

Department of Pediatrics,
Johns Hopkins University School of Medicine,
Baltimore, Maryland 21205

Received 24 March; accepted 30 June 1977.

- ¹ Glynn, J. M. *Pharmacol. Revs.* **16**, 381-407 (1964).
- ² Baker, R. M. in *Biochemistry and Turnover of Membrane Macromolecules* (ed. Cook, J. S.) 93-103 (Raven, New York, 1976).
- ³ Mayhew, E. J. *Cell Physiol.* **79**, 441-451 (1972).
- ⁴ Baker, R. M. *et al.* *Cell* **1**, 9-21 (1974).
- ⁵ Mankovitz, R., Buchwald, M. & Baker, R. M. *Cell* **3**, 221-226 (1974).
- ⁶ Corsaro, C. M. & Migeon, B. R. *Expl Cell Res.* **95**, 47-53 (1975).
- ⁷ Gilula, N. B., Reeves, O. R. & Steinbach, A. *Nature* **235**, 262-265 (1972).
- ⁸ Azarmi, R., Larsen, W. J. & Loewenstein, W. R. *Proc. natn. Acad. Sci. U.S.A.* **71**, 880-884 (1974).
- ⁹ Gilula, N. B. in *Cell Communication* (ed. Cox, R. P.) 1-29 (New York, 1974).
- ¹⁰ Pinto da Silva, P. & Martinez-Palomo, A. *Proc. natn. Acad. Sci. U.S.A.* **72**, 572-576 (1975).
- ¹¹ Fushpan, E. J. & Potter, D. D. *Curr. Top. dev. Biol.* **3**, 95-127 (1968).
- ¹² Cox, R. P., Krauss, J. R., Balis, M. E. & Dancs, J. *Expl Cell Res.* **74**, 251-268 (1972).
- ¹³ Pitts, J. D. in *Cell Interactions* (ed. Silvestri, L. G.) 277-285 (American Elsevier, New York, 1972).
- ¹⁴ Corsaro, C. M. & Migeon, B. R. *Expl Cell Res.* **95**, 39-46 (1975).
- ¹⁵ Bennett, M. V. L. *Fedn Proc.* **32**, 65-75 (1973).
- ¹⁶ Sheridan, J. D. in *The Cell Surface in Development* (ed. Moscona, A. A.) 187-206 (Wiley, New York, 1974).
- ¹⁷ Loewenstein, W. R. in *The Nervous System* (ed. Tower, D. B.) 419-436 (Raven, New York, 1975).
- ¹⁸ Simpson, L., Rose, B. & Loewenstein, W. R. *Science* **195**, 294-296 (1977).

Synergism between anti-microtubule agents and growth stimulants in enhancement of cell cycle traverse

COLCHICINE and other mitotic inhibitors have been used widely in cell cycle studies to produce synchronous cell populations¹. This application is based on the ability of these drugs to inhibit mitosis by binding to tubulin and preventing microtubule (mitotic spindle) formation². As well as inhibiting mitosis, these drugs modify the morphology and functions of cells during interphase. For example, colchicine inhibits the formation of cilia³, affects phagocytosis, the normal distribution of lysosomes⁴, and the synthesis and secretion of various proteins⁵⁻⁷ and alters the transport of certain nucleotides⁸. At very high concentrations ($2-35 \times 10^{-3}$ M) colchicine and colcemid retard the initiation of DNA synthesis and prolong the period of DNA synthesis⁹. We describe here the synergistic effects of colchicine and other anti-microtubule agents at lower concentrations ($1-2 \times 10^{-7}$ M) in the insulin- or serum-induced increase in cells moving from G₁ to S phase. Our results suggest that microtubules have an important role in the regulation of DNA synthesis by growth factors.

High density chick cells starved of serum can be stimulated synchronously by insulin to traverse at least one cell cycle¹⁰. This stimulation was detected as an increase in the proportion of cells in S phase with 8-10-h lag when the increase in DNA per cell was detected by flow microfluorometry (FMF). When similar experiments were performed in the presence of 2×10^{-7} M colchicine, it was evident that colchicine significantly increased the population in the S phase of the cell cycle (Fig. 1). The stimulated cells progressed through S phase more slowly than in the absence of colchicine, but the degree of synchrony was as good as the stimulated population when insulin was used alone.

Two other anti-microtubule agents, colcemid and vinblastines were compared with colchicine in their ability to affect the proportion of cells stimulated to make DNA in insulin-treated chick cell cultures. All of these compounds increased the cell population moving out of G₁ phase in the presence of insulin (Table 1). Colchicine and vinblastine were more effective than colcemid at lower concentrations. Furthermore, the continuous presence of anti-microtubule agents was not necessary, for their removal from the media after 6 h had the same effect (data not shown). These observations suggest that the primary effect of anti-microtubule agents at low concentrations is on the early events in the stimulation of DNA synthesis. At higher concentrations ($> 5 \times 10^{-7}$ M), all of these compounds decreased the number of cells engaged in DNA synthesis, probably due to toxic side effects. Thus the mode of action of these drugs is different when compared at low and high concentrations.

To determine whether or not this stimulatory effect also applied to cell lines, the effect of anti-microtubule agents on BALB 3T3 cells was compared with that on chick fibroblasts. In these experiments cultures at their saturation density were stimulated with fresh medium containing either insulin or serum. The proportion of cells in the S+G₂+M phases with or without added growth stimulants are listed in Table 2. As before, we found that insulin had little or no stimulatory effect on DNA synthesis in BALB 3T3 A31 cells. This was true even when the insulin concentration was raised to 128 mIU ml⁻¹. Similar results were obtained with Don hamster cells at high density (results not shown). Furthermore, only chick cells could be stimulated to synthesise DNA by fresh medium. It is important to note that colchicine was effective in stimulating cell movement from G₁ to S only

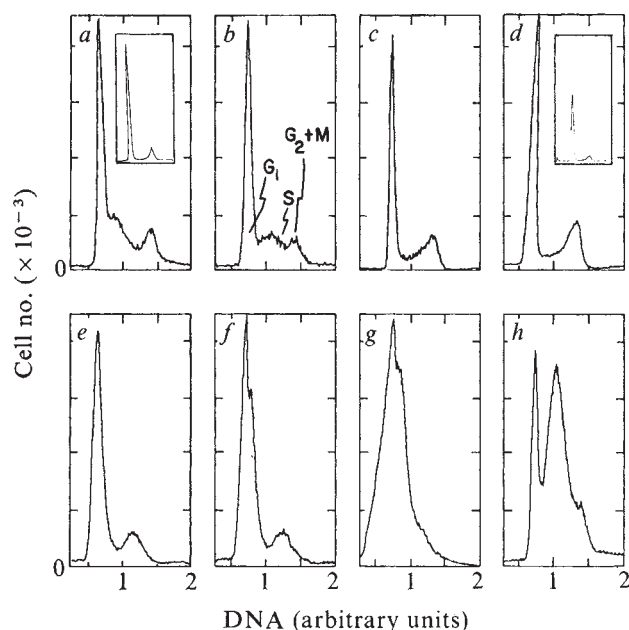


Fig. 1 Progress of chick embryo fibroblasts through the cell cycle in the presence of insulin and colchicine. Confluent secondary chick embryo fibroblasts in medium 199 were prepared and deprived of serum as described before¹⁰. After serum starvation, insulin was added at a concentration of 16 mIU per ml medium. Parallel cultures were provided with insulin and 2×10^{-7} M colchicine and were incubated in 5% CO₂ at 39 °C. Cells were collected and prepared for FMF analysis, using propidium iodide as the fluorescent stain²⁵. FMF patterns of insulin-treated cultures are a-d for the time points of 13, 17, 23 and 29 h respectively, and insulin plus colchicine are e-h for the same time sequence. The inserted histograms in (a) and (d) are control cultures in medium 199 at 0 and 29 h. Histograms were plotted to give the peak channel at approximately the same height. The number of cells ($\times 10^3$) in the peak channel was a, 2.7; b, 1.0; c, 1.3; d, 5.5; e, 4.0; f, 2.2; g, 4.0 and h, 3.5.

when the primary growth stimulant, a mitogen, was able to stimulate some cell cycle traverse. Therefore, colchicine and other anti-microtubule agents act as 'synergistic agents' to a 'mitogen'.

How does the message for DNA synthesis get transferred from the mitogen to the cell? The cell membrane is believed to have an important role in such progress. Some growth stimulants have been partially purified from various sources, and shown to bind to the surface membrane of cultured cells¹¹⁻¹³. What occurs between the binding of growth stimulants and their relation to the initiation of DNA synthesis is, however, poorly understood.

The dynamics of the membrane receptors and other components seem to be subject to controls from both sides of the membrane. For example, concanavalin A (con A)

Table 1 Effect of colchicine, colcemid and vinblastine on cell cycle traverse of insulin-treated chick embryo fibroblasts

Concentrations of mitotic inhibitors (M)	% of cells in S+G ₂ +M phases		
	Colchicine	Colcemid	Vinblastine
0 (insulin only)	25	25	25
5×10^{-8}	47	27	58
1×10^{-7}	60	25	64
2×10^{-7}	69	54	60
5×10^{-7}	64	62	63

Cells were grown and prepared for FMF analysis as for Fig. 1. Colcemid, vinblastine, and colchicine were tested at the concentrations indicated. Insulin was present in all cultures. Cells were collected 22 h after addition of mitotic inhibitors and insulin. After FMF analysis, the % population in S+G₂+M phases were calculated with a computer program.

and other related plant lectins inhibit the immunoglobulin capping on lymphocytes^{14,15}. Con A by itself can form caps on various cell types and the process is either inhibited or stimulated by anti-microtubule or anti-microfilament agents depending on cell types studied¹⁵⁻¹⁸. But, the evidence for the involvement of microtubules in growth regulation has been of a 'negative' nature so far, that is, agents affecting these organelles would inhibit the stimulatory action caused by a mitogen⁹. Most of these studies were carried out at high concentrations of inhibitors, where possible toxic side effects could have complicated the interpretation of the results. Our evidence of the positive effect of anti-microtubule agents on mitogen-stimulated DNA synthesis directly demonstrates involvement of microtubules in growth regulation.

The presence of insulin receptors on chick fibroblasts has been demonstrated in detail¹⁹. Furthermore, site-site interaction and redistribution of insulin receptors after the binding of insulin to the membrane of rat liver or human lymphocytes have been reported²⁰. The striking synergistic effect of colchicine and insulin on stimulation of DNA synthesis in chick fibroblasts reported here makes it possible to propose the following model to explain such synergism (Fig. 2). In this model which is a modification of that proposed by Nicolson²¹ and by McGuire

Table 2 The effect of colchicine and growth stimulants on cell cycle traverse

Cell types and media	% of cells in S+G ₂ +M phases	
	-Colchicine	+Colchicine
Secondary chick embryo fibroblasts		
Medium 199 (control)	14	32*
+insulin, 16 mIU ml ⁻¹	29	66
+chick serum, 3%	33	58
BALB 3T3 A31		
Medium DME (control)	4	4
+insulin, 128 mIU ml ⁻¹	4	4
+calf serum, 10%	15	29
+calf serum, 20%	30	78
+calf serum, 30%	33	90

Data for secondary chick embryo fibroblasts were obtained as described in Table 1. Values are averages of two experiments. BALB 3T3 A31 cells were grown to confluency in medium DME containing 10% calf serum for 5 d. Cells were washed with saline and provided with fresh medium DME containing additional components indicated. After 28 h, cells were collected and prepared for FMF analysis.

*The degree of stimulation on cells in medium 199 (control) varied with each experiment depending on the batch of cells.

and Barber²², a growth stimulant after binding activates its receptor on the membrane by forming an active stimulant-receptor complex. To complete a signal for DNA synthesis, however, cooperation of this complex with certain membrane components must take place. If the mobility of membrane receptors and second components is an important factor in the signal transfer, any compound which increases this mobility should facilitate DNA synthesis. Microtubules have been proposed to be an anchorage agent for certain membrane components^{14,21}. Our results indicate that this may indeed be the case. A factor which destroys microtubule structure, colchicine in this case, can be a synergistic agent for growth stimulation. Such an agent by itself is incapable of triggering DNA synthesis. It acts only when some DNA synthesis capacity is already present.

It has been reported that BALB 3T3 cells indeed have insulin receptors²³. But binding of insulin to these receptors fails to stimulate DNA synthesis in these cells (ref. 10, and the results here). In addition, the cell line of this type and human diploid foreskin or ovine embryonic trachea fibroblasts fail to show growth stimulation on addition of trypsin, an agent which stimulates growth in secondary

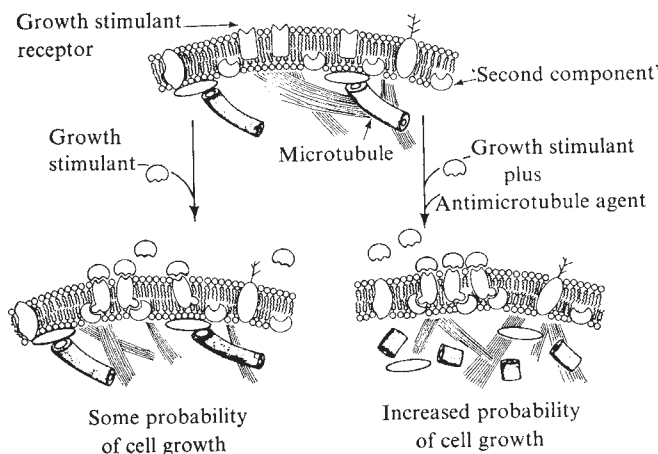


Fig. 2 Schematic representation of message transfer. The inactive receptor for 'growth stimulant (mitogen)' and 'second component' for carrying on message transfer are distributed randomly on the membrane, and their mobilities are controlled by membrane fluidity which in turn, is influenced by the cytoplasmic microtubules along the inner side of the membrane. When anti-microtubule agents are present, the cytoplasmic microtubules are depolymerised and the mobilities of membrane components increase. Thus, the chance of interaction between the activated receptor complex and the second component is higher and the message for DNA synthesis is transferred much more efficiently.

chick fibroblasts²⁴. The most likely explanation is that there is fundamental difference in the nature of membranes of primary cell cultures and cell lines. It is possible that the interaction between mitogen receptor complex and second membrane component is blocked in cell lines unless a more complicated factor such as selenium is provided to potentiate the cooperation. Alternatively, the activation of certain mitogen receptors, for example, insulin receptors, may not occur after the binding of the mitogen. Therefore, no message is generated and transferred to the second component. There is an additional possibility that the second membrane component for message transfer from particular single growth factor may be altered in cell lines. As a result, the growth control of cell lines in culture becomes much more stringent than that of primary cell cultures as discussed previously¹⁰. The exact nature of the synergistic effects of anti-microtubule agents and growth stimulants is currently under investigation.

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MEI-HUI TENG
JAMES C. BARTHOLOMEW
MINA J. BISSELL

Laboratory of Chemical Biodynamics,
Lawrence Berkeley Laboratory,
University of California,
Berkeley, California 94720

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- 1 Stubblefield, E. & Klevecz, R. *Expl Cell Res.* **40**, 660-664 (1965).
- 2 Wilson, L., Bamberg, J. R., Mizel, S. B., Grisham, L. M. & Crestwell, K. M. *Fedn Proc.* **33**, 158-166 (1974).
- 3 Stubblefield, E. & Brinkley, B. R. *J. Cell Biol.* **30**, 645-652 (1966).
- 4 Robbins, E. & Gonatas, N. K. *J. Histochem. Cytochem.* **12**, 704-711 (1964).
- 5 Stein, O., Sanger, L. & Stein, Y. *J. Cell Biol.* **62**, 90-103 (1974).
- 6 Le Marchand, Y., Patzelt, C., Assimacopoulos-Jeanet, F., Loten, E. G. & Jeanrenaud, B. *J. clin. Invest.* **53**, 1512-1517 (1974).
- 7 Rojkind, M. & Kershenovich, D. *Biochim. biophys. Acta* **378**, 415-423 (1975).
- 8 Mizel, S. B. & Wilson, L. *Biochemistry* **11**, 2573-2578 (1972).
- 9 Fitzgerald, P. H. & Brehaut, L. A. *Expl Cell Res.* **59**, 27-31 (1970).
- 10 Teng, M., Bartholomew, J. C. & Bissell, M. J. *Proc. natn. Acad. Sci. U.S.A.* **73**, 3173-3177 (1976).

- 11 Van Obberghen, E., De Meyts, P. & Roth, J. *J. biol. Chem.* **251**, 6844-6851 (1976).
- 12 Hollenberg, M. D. *Archs Biochem. Biophys.* **171**, 371-377 (1975).
- 13 Carpenter, G. & Cohen, S. *J. Cell Biol.* **71**, 159-171 (1976).
- 14 Yahara, I. & Edelman, G. M. *Ann. N.Y. Acad. Sci.* **253**, 455-469 (1975).
- 15 Aubin, J. E., Carlsen, S. A. & Ling, V. *Proc. natn. Acad. Sci. U.S.A.* **72**, 4516-4520 (1975).
- 16 De Petris, S. *Nature* **250**, 54-55 (1973).
- 17 Edelman, G. M., Yahara, I. & Wang, J. L. *Proc. natn. Acad. Sci. U.S.A.* **70**, 1442-1446 (1973).
- 18 Mintz, U. & Sachs, L. *Proc. natn. Acad. Sci. U.S.A.* **72**, 2428-2432 (1975).
- 19 Raizada, M. K. & Perdue, J. F. *J. biol. Chem.* **251**, 6445-6455 (1976).
- 20 De Meyts, P., Bianco, R. & Roth, J. *J. biol. Chem.* **251**, 1877-1888 (1976).
- 21 Nicolson, G. L. *Biochim. biophys. Acta* **451**, 57-108 (1976).
- 22 McGuire, R. F. & Barber, R. J. *Supramolec. Struct.* **4**, 259-269 (1976).
- 23 Thomopoulos, F., Roth, J., Lovelace, E. & Pastan, I. *Cell* **8**, 417-423 (1976).
- 24 Cunningham, D. D. & Ho, T. in *Cold Spring Harbor Conferences on Cell Proliferation* 2 (eds Reich, E., Rifkin, D. B. & Shaw, E. E.) 795-806 (Cold Spring Harbor Laboratory, 1975).
- 25 Crissman, H. A. & Steinkamp, J. A. *J. Cell Biol.* **59**, 766-771 (1973).

5-Hydroxytryptamine and punishment

PUNISHMENT is defined as the presentation of a noxious stimulus and results in the suppression of behaviour. This suppressive effect of punishment is seen clearly in conflict situations where the organism is encouraged to respond and then is punished for doing so. Brain 5-hydroxytryptamine (5-HT) systems have been implicated in the processes associated with the response to punishment. We show here that selective neurotoxin lesions to the 5-HT forebrain pathways reverse the suppressive effects of punishment, as do minor tranquillisers, supporting the view that 5-HT has a central role in conflict behaviour and in the effect of minor tranquillisers on such behaviour.

If a response is followed by punishment, the probability of its occurrence decreases. 5-HT antagonists¹⁻³ and synthesis inhibition⁴⁻⁶ have been shown to re-instate rates of punished responding. Furthermore, behavioural release following synthesis inhibition has been shown to correlate closely with the pharmacological time course of 5-HT depletion⁶. Qualitatively similar behavioural effects have been reported with anxiolytic drugs such as the benzodiazepines and with pharmacological studies in support, it has been suggested that at least part of the anti-anxiety action of these drugs is due to decreased 5-HT functional activity⁷. But, the lack of neuropharmacological specificity associated with the existing methods for reducing 5-HT activity makes clear experimental investigation of this hypothesis difficult.

The neurotoxic agent, 5,7-dihydroxytryptamine can be injected directly into the brain to destroy 5-HT neurones. Stein and colleagues⁸ have reported a profound, although transitory, release of punished behaviour in a conflict paradigm, accompanied by little effect or unpunished responding, following intraventricular injection of 5,6-dihydroxytryptamine (5,6-DHT). Neurotoxic indoleamines have been injected into specific brain areas⁹ and the balance of neurochemical data (for instance ref. 10) suggests that 5,7-DHT produces minimal nonspecific degeneration. Therefore, intracerebral injections of 5,7-DHT were used to provide more direct evidence for a role of 5-HT in punishment processes.

Male Sprague-Dawley rats (250-300 g) were trained to press a lever for food reward on a continuous reinforcement (CRF) schedule. Following this they were randomly divided into three groups. 5,7-DHT creatinine sulphate (Regis) ($n = 8$, 2 μ g per 2 μ l free base, on each side of brain) or vehicle ($n = 5$, 1 mg ml⁻¹ ascorbic acid in sterile isotonic saline) was injected (1 μ l min⁻¹) bilaterally into the ventromedial tegmentum. Operated control animals ($n = 5$) underwent the same procedure except that the injection needle was not lowered into the brain. This latter group was included to assess any behavioural disturbances due to 5-HT depletion in vehicle injected animals observed in an earlier pharmacological study. Ether anaesthesia was used and all animals received desmethylimipramine (25 mg per kg body weight, intraperitoneally, since this prevents the effects of 5,7-DHT on NA neurones)^{11,12} 45 min before surgery. The incisor

bar was set 3.2 mm above the intra-aural plane and coordinates taken from the lambda (+1 mm) skull suture (caudal, 6.5 mm; ventral, 11.3 mm; lateral, 0.5 mm; injection needle introduced at an angle of 47° to the vertical plane).

After surgery, free-feeding weight was reduced to 85% and the effects of the surgical treatments were examined on the acquisition of a multiple three component schedule involving two reward components and a time-out period. The three components were signalled by different light conditions—during the first reward component (10 min) the illumination was provided by the house light alone, time-out (4 min) was signalled by darkness; and during the second reward component (4 min) both the house light and lever lights were on. This sequence, reward (1), time-out, reward (2) was presented twice during each testing session. Reinforcement in the reward components was available on a variable interval (VI) 25 s (with limits of 5 and 55 s) schedule. No reinforcement was programmed during time-out. Animals were tested on this schedule for 12 consecutive days, (training days 1–12).

During the following 6 d (days 13–18) the second reward component was changed to a conflict situation—reinforcement was again available on the same VI schedule. Each reinforced response, however, was also punished (0.8 mA electric shock of 0.5 s to the feet).

Mean responses per minute over successive training days 1–12 for each surgical group in the reward (1) and time out components are shown in Fig. 1. Although lesioned animals tended to respond at a lower rate no overall significant differences were observed between groups during 12 d of acquisition training on the two reward components of the multiple schedule (Kruskal-Wallis, reward (1) $H = 2.62$, $P > 0.20$; reward (2), $H = 4.45$, $P > 0.10$). Furthermore rates of responding in these two components did not differ within each surgical group. But a similar analysis showed a significant overall difference between groups during acquisition of the time-out component ($H = 13.79$,

Fig. 1 Response per minute for each surgical group (C, control; V, vehicle; L, lesion) during the training days 1–12 (postoperative days 11–23). ▲, Responding during reward (1); ●, responding during time-out component. The three experimental groups are denoted with different line markings.

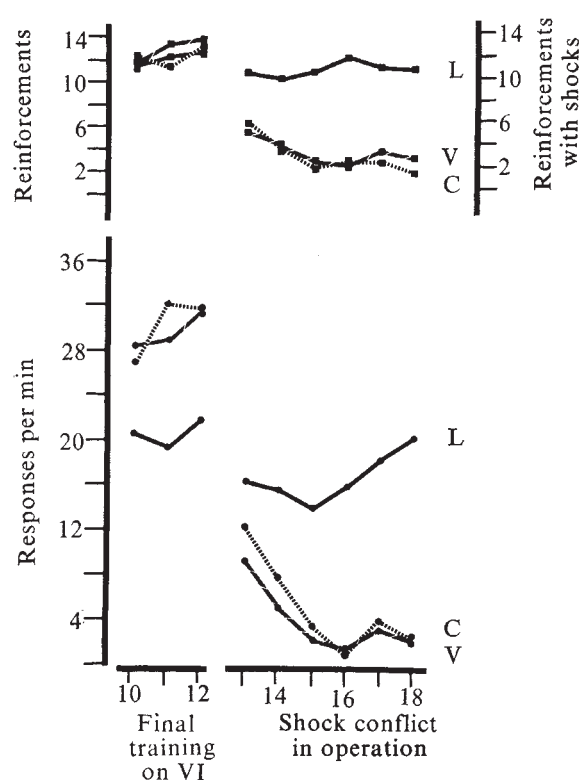
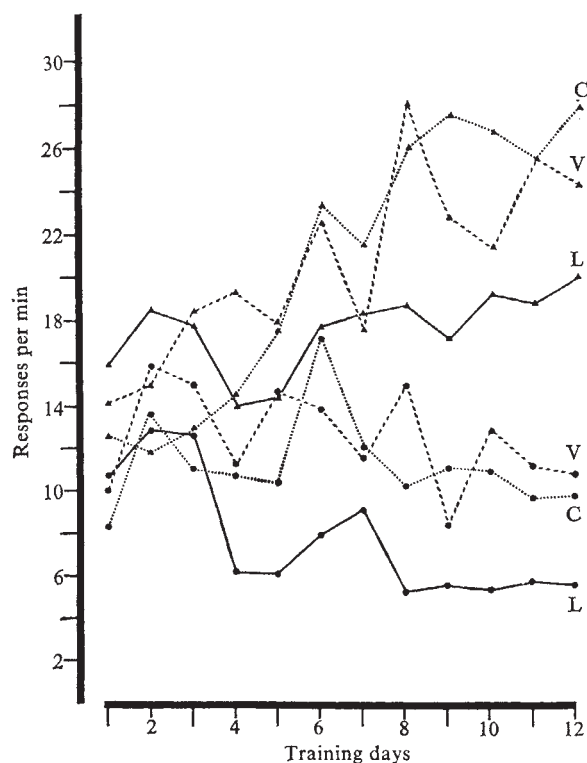


Fig. 2 Third schedule component. Left axis, responses per min and mean reinforcements received for each surgical group (during reward component 2) on the final three training days. Right axis, responses per min (●) and mean reinforcements plus shocks (■) during reward component 2 (now conflict component) for each surgical group (C, control; V, vehicle; L, lesion) on the 6 days of conflict following acquisition training. The experimental groups are denoted with different line markings.

$P < 0.01$). Inspection of the data showed that this was due to less responding by the lesioned animals.

The effects of shock introduction on response rate and shock acceptance in the former reward (2) component are shown in Fig. 2. This manipulation resulted in a dramatic decrease in response rate in both the control and vehicle groups. No suppression was observed in the lesioned animals, however, and correspondingly these animals accepted more shocks. These differences were highly significant overall (responses, $H = 11.56$, $P < 0.01$; shocks, $H = 12.38$, $P < 0.01$) and were observed until the end of behavioural testing on post-operative day 77. No significant overall differences were observed during reward (1) ($H = 3.66$, $P > 0.10$) although lesioned animals continued to respond at a lower rate during time-out ($H = 6.68$, $P < 0.05$).

For biochemical analysis animals were killed on post-operative days 97 and 98 and amine uptake into cortex slices and hypothalamic synaptosomal preparations determined^{13,14}. With respect to controls, lesioned animals showed highly significant reductions in 5-HT uptake both in the cortex (77%) (Mann-Whitney test $P < 0.002$) and hypothalamus (66%) ($P < 0.004$) while the vehicle group showed much smaller non-significant reductions (cortex, 25%; hypothalamus, 7.5%). Uptake of noradrenaline in the cerebral cortex was not significantly altered (Mann-Whitney, $P > 0.8$) in any group of animals (vehicle 11%, 5,7-DHT 15% increases of sham). Uptake of hypothalamic noradrenaline was not measured in these experiments but identical 5,7-DHT lesions in a separate series had no significant effect on this parameter, while producing the same pattern and degree of 5-HT denervation¹⁵. Everitt (in preparation) has also shown no changes in dopamine uptake in septum or striatum after 5,7-DHT lesions in the MFB. Histological analysis of the lesion procedure has shown the canula placement to be localised in the decussation of the brachium conjunctivum dorsal to the interpeduncular nucleus.

Taken together these findings provide strong support for the

postulation of a 5-HT 'punishment' system that is critically involved in the acquisition of behavioural suppression in conflict situations. In other experiments, previously acquired suppression was also found to be released by the 5,7-DHT lesion. The punishment-releasing effect of 5-HT depletions does not seem to reflect a nonspecific concomitant of a general release syndrome since the lesioned animals did not show higher rates of responding during the other schedule components. Reduced effectiveness of the electric shock in suppressing responding could be attributed to a reduced pain sensitivity. This is an unlikely interpretation of the results, however, as morphine, a classic analgesic does not re-instate shock-suppressed responding¹⁶ and Hole¹⁷ has reported that 5,7-DHT injections at sites in the midbrain similar to those used here have no effect on pain sensitivity.

It would be interesting to establish if chlordiazepoxide induces characteristic behavioural effects in 5,7-DHT lesioned rats. The most important of these effects is the ability of the minor tranquilisers to reinstate responding suppressed by punishment. But, this is clearly difficult to investigate when, as we have shown, the 5,7-DHT lesion itself releases punished responding. Rates of responding during conflict should be equivalent in all groups of animals before the effects of an anxiolytic drug are evaluated. Preliminary investigations of this question were conducted on the present animals, when the initial conflict experiment and a study of the effects of amphetamine on this behaviour had been completed. On postoperative days 31–34 the animals were re-stabilised on the basic three-component conflict schedule. During the next 12 d the shock level during the conflict component was increased from 0.8 mA to 2.5 mA in four steps (1.3, 1.6, 2.0, 2.5 mA). The effects of chlordiazepoxide (1.26, 2.0 mg per kg) were studied. The increased shock level decreased significantly conflict responding in the lesioned animals. Chlordiazepoxide did not re-instate responding in these animals to the same degree seen in the control animals. These results may reflect a block of CDP action in the 5,7-DHT-lesioned rats. Caution must be exercised, however, because the lesioned animals were responding in conflict at a higher rate than controls despite the raised shock level. It is therefore possible that under CDP the lesioned rats reach their highest possible rate of responding sooner and thus a response-releasing effect of the normal magnitude was not possible. But it is tempting to speculate that 5-HT pathways have a central role in mediating both the effects of shock on responding and the reversal of these effects with benzodiazepines.

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N. C. TYE

B. J. EVERITT

SUSAN D. IVERSEN

*The Psychological Laboratory
and Department of Anatomy,
University of Cambridge,
Cambridge, UK*

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- ¹ Graeff, F. G. & Schoenfeld, R. I. *J. Pharm. exp. Ther.* **173**, 277–283 (1970).
- ² Stein, L., Wise, C. D. & Berger, B. D. in *The Benzodiazepines* (eds Garattini, S., Mussini, G. & Randall, L. D.) (Raven, New York, 1973).
- ³ Geller, I., Hartmann, R. J. & Croy, D. J. *Res. Commun. Chem. Pathol. Pharmac.* **7**, 165–174 (1974).
- ⁴ Geller, I. & Blum, K. *Eur. J. Pharmac.* **9**, 319–324 (1970).
- ⁵ Robichand, R. C. & Sledge, K. L. *Life Sci.* **8**, 965–969 (1970).
- ⁶ Wise, C. D., Berger, B. D. & Stein, L. *Biol. Psychiat.* **6**, 3–21 (1973).
- ⁷ Wise, D. C., Berger, B. D. & Stein, L. *Science* **177**, 180–183 (1972).
- ⁸ Stein, L., Wise, D. C. & Belluzzi, J. D. *Adv. biochem. Psychopharmac.* **14**, (1975).
- ⁹ Bjorklund, A., Nobin, A. & Stenavi, U. *Anatomia* **145**, 489–501 (1973).
- ¹⁰ Baumgarten, H. G. et al. *J. Neurochem.* **19**, 1587–1597 (1972).
- ¹¹ Bjorklund, A., Baumgarten, H. G. & Renach, D. *J. Neurochem.* **24**, 833–835 (1975).
- ¹² Gerson, S. & Baldessarini, R. J. *Brain Res.* **35**, 140–145 (1975).
- ¹³ Lidbrink, P., Jonsson, G. & Fuxe, K. *Neuropharmacology* **10**, 521–536 (1971).
- ¹⁴ Sachs, C. & Jonsson, G. *Res. Commun. Chem. Pathol. Pharmac.* **4**, 203–220 (1972).
- ¹⁵ Everitt, B. J., Fuxe, K. & Jonsson, G. *J. Pharmac. (Fr.)* **6**, 25–32 (1975).
- ¹⁶ Kelleher, R. T. & Morse, W. H. *Fedn Proc.* **23**, 808–817 (1964).
- ¹⁷ Hole, K. *Brain Res.* **107**, 385–399 (1976).

Intranigral kainic acid is evidence that nigral non-dopaminergic neurones control posture

THE substantia nigra has a key role in the control of posture and in the coordination of movements. This function is currently attributed to the dopaminergic neurones ascending from the pars compacta of the nigra to the neostriatum. In fact unilateral lesion of this pathway by intranigral injection of 6-hydroxydopamine (6OH-DA) produces a dramatic asymmetry consisting of a tendency of the animal to turn towards the side of the lesion (ipsilateral turning)¹. But, unilateral electrolytic lesions of the substantia nigra, while similarly effective to 6OH-DA in destroying dopaminergic neurones, result in turning towards the intact side (contralateral turning)^{2,4}. This has been taken to indicate the existence of non-dopaminergic neurones arising from or traversing the nigra, which control turning behaviour in a manner opposite to the nigro-neostriatal dopaminergic neurones^{2,4}. We report here that unilateral intranigral injection of kainic acid^{5,6} produces a sustained contralateral turning independent from dopaminergic nigro-striatal function. Since kainic acid spares axons but produces neuronal loss in the pars reticulata of the nigra, our results indicate the existence of non-dopaminergic neurones originating in the pars reticulata which control turning behaviour and posture in a manner opposite to the nigrostriatal dopamine (DA) neurones.

Male Sprague-Dawley rats (290–310 g) were anaesthetised with Equithesin (3.5 mg per kg) and placed in a Stellar Stoelting stereotaxic apparatus; kainic acid (in saline) or 6OH-DA (8 µg of base in saline with 0.1% ascorbic acid) were injected in the substantia nigra using stereotaxic coordinates A-3, L 2, V 7.8, according to the Pellegrino and Cushman⁷. The total injection volume was 0.3 µl, injected at a constant rate of 1 µl per 18 min, through a 0.25-mm stainless-steel cannula. Unless specified, kainic acid was injected in the nigra at the dose of 0.75 µg. Twenty-four hours after intranigral kainic acid the intensity of turning behaviour was assessed as described in the legend to Table 1. Ten days after intranigral kainic acid 42 rats were killed, the brain perfused with formalin, included in paraffin and the mesencephalon cut into serial 20-µm slices which were stained with Luxol fast-cresylviolet in order to ascertain the location of the cannula tip and the extension of the lesion. In another series of rats, injected with kainic acid 10 d previously, the nigra of each side was punched out with stainless-steel cannulas from two 400-µm slices made by cryostat; glutamic acid decarboxylase (GAD), tyrosine hydroxylase (TH) and choline-acetyltransferase (CAT) were assayed according to McGeer and McGeer⁸ with slight modifications.

Figure 1 shows the histological appearance of the substantia nigra injected with kainic acid 10 d previously as compared with that injected with saline, in sections stained with cresylviolet–Luxol fast blue. In the lesioned nigra the pars reticulata reveals moderate loss of neuronal perikarya and gliosis, characterised by proliferation of non-neuronal elements. The pars compacta seems less affected than the pars reticulata and still shows the presence of numerous large globose neurones. There are no signs of demyelination in or around the substantia nigra.

Intranigral injection of kainic acid produces homolateral turning which appears as soon as the animals recover from the anaesthesia and dominates the acute stage of the symptomatology, lasting about 6–12 h. As the ipsilateral turning subsides rats undergo a variable phase of intermittent ipsi- and contralateral turning but by 24 h after kainic acid administration they show an asymmetric posture with turning contralateral to the lesion.

As shown in Table 1, the intensity and the incidence of the contralateral turning is related to the dose of kainic acid, being maximal after 1.0 µg.

The contralateral turning is maximal during the first 3–4 d

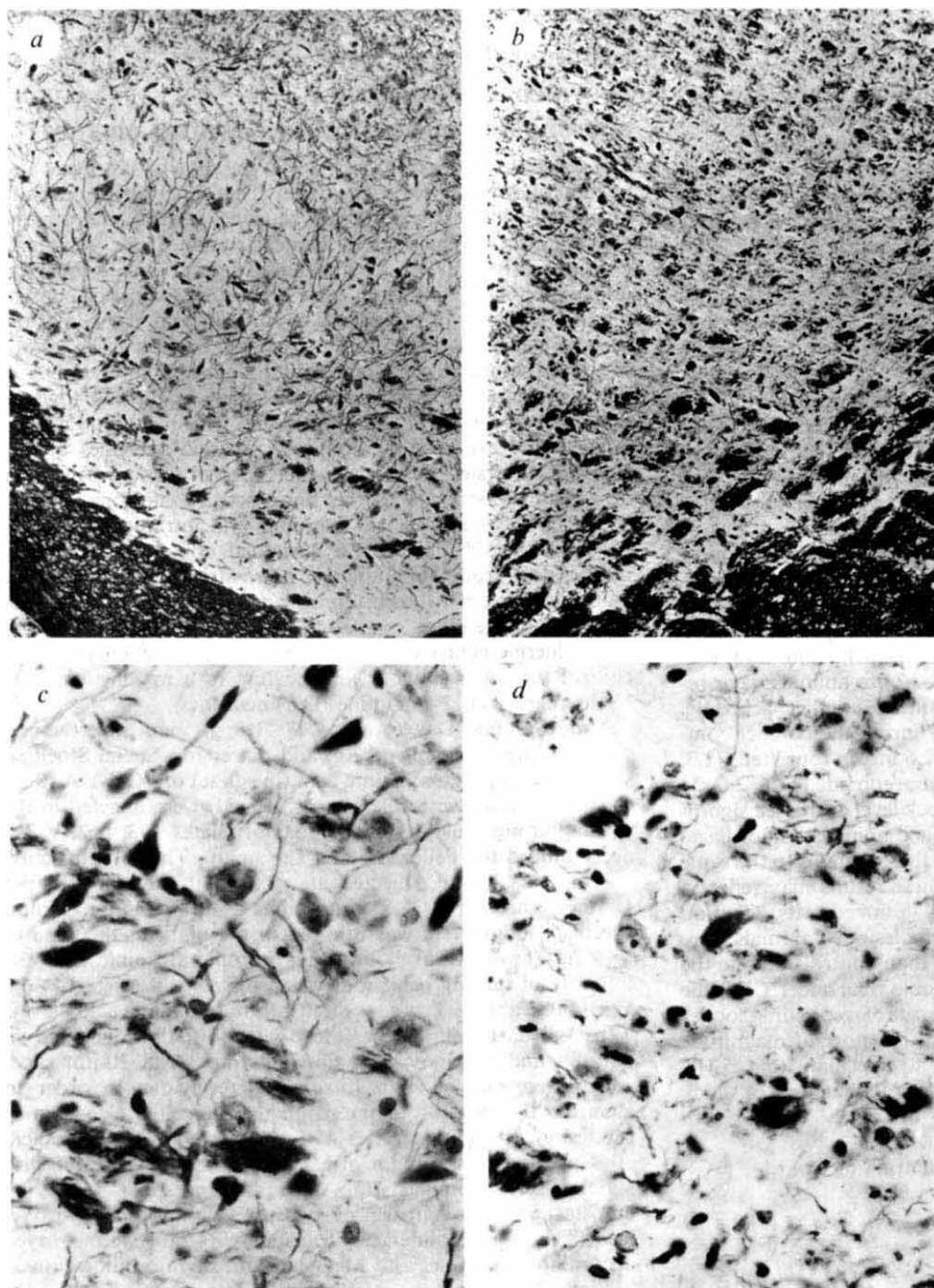


Fig. 1 Micrographs of Luxol fast-cresylviolet stained sections of substantia nigra pars reticulata from a rat injected unilaterally in the nigra with 0.75 μ g of kainic acid, 10 d previously. *a, c*, Control side; *b, d*, kainic-injected side. *a*, And *b*, $\times 5$; *c* and *d*, $\times 20$.

after kainic administration and, although diminished in intensity, is still present 10 d later. Failure to obtain the chronic contralateral turning was associated, in 10 out of 12 rats, with placement of the cannula tip outside the substantia nigra. Among 30 rats where contralateral turning was obtained, the tip of the cannula was found to be placed in the centre of the pars reticulata in 16, at the border between the compacta and the reticulata in 8, in the pars lateralis in 4 and in the medial part of the nigra in 2 cases. Ten days after intranigral kainic acid nigral GAD and CAT in the kainic-injected side are not significantly different from the contralateral one while nigral TH is decreased by about 30% in the kainic-injected side (GAD: 965 ± 45 compared with 987 ± 52 ; CAT: 8.8 ± 0.8 compared with 8.3 ± 0.6 ; TH: 11.8 ± 0.6 compared with 8.2 ± 0.5 ; means $\pm$ s.e.m. of 10 determinations in control compared with kainic-injected side expressed in nmol per h per mg protein).

Bilateral intranigral administration of 6OH-DA, which decreased nigral TH-activity by more than 85% in respect to controls (see legend to Table 1), failed to affect the induction

of chronic contralateral turning by kainic acid injected unilaterally in the substantia nigra (see Table 1).

Our results show that unilateral intranigral administration of kainic acid produces chronic contralateral turning. This effect was independent from an action on nigro-neostriatal DA-neurons, as it took place also when they had been previously destroyed bilaterally by intranigral 6OH-DA. Kainic acid produced a distinct loss of nigral neurones but did not damage myelinated fibres or change the activity of nigral GAD, a marker of afferent GABA-connections⁸⁻¹⁰. This indicates that the contralateral turning produced by intranigral kainic acid is due to destruction of nigral neurones rather than to damage of fibre tracts afferent to the nigra or passing near it.

Since the chronic effects of the kainic-induced nigral lesion are just opposite to the selective destruction of the nigrostriatal DA-neurons with 6OH-DA and are in turn unrelated to their intactness, our results indicate the presence, in the substantia nigra, of neurones controlling turning behaviour and posture in a manner opposite to the dopaminergic ones. Stimulation of

Table 1 Incidence and intensity of contralateral turning after various doses of intranigral kainic acid

Kainic acid (μ g)	6OH-DA (8 μ g)	Rats injected	Rats turning with score				Turns per 5 min*
			0	1	2	3	
0.25	—	10	8	2			—
0.50	—	15	5	2	7	1	15
0.75	—	30	6	2	6	16	32 $\pm$ 5
0.75	+	20	3	1	4	12	35 $\pm$ 7
1.0	—	20	1	1	4	14	38 $\pm$ 6

Turning was scored 24 h after unilateral intranigral kainic acid according to Costall *et al.*⁴. 6OH-DA was administered in the nigra of both sides 15 d before kainic acid. In these rats, nigral TH was decreased by about 87% in respect to controls (1.5 ± 0.4 compared to 12.2 ± 2.5 , mean $\pm$ s.e.m. of five determinations).

*Values are mean $\pm$ s.e.m.

these non-DA neurones by kainic acid would explain the homolateral turning obtained in the acute stage after intranigral kainic acid.

The chronic effects of the intranigral administration of kainic acid are strikingly similar to those obtained by electrocoagulative lesions of the nigra³⁻⁴. This suggests that the effects of the electrolytic lesions are due to destruction of nigral neurones rather to damage of fibre tracts.

As to the nature of the neurones destroyed by kainic acid, the fact that the loss of nigral perikarya is confined to the pars reticulata and that CAT activity is within the normal range, suggests that the lesion produced by kainic acid is limited to a fairly specific neuronal population. Indeed this would not be unexpected since the toxic effect of kainic acid, being related to stimulation of specific glutamate receptors¹¹, should vary among different neuronal types depending on the density of glutamate receptors on their perikarya. Accordingly, the relative intactness of the pars compacta and the minor reduction of nigral TH after intranigral kainic acid as compared with the dramatic decrease of striatal GAD and CAT after intrastriatal kainic acid^{5,6}, suggests that the dopaminergic nigral perikarya are much less sensitive to the toxin than striatal GABA-ergic and cholinergic perikarya.

The histological procedure adopted in this study does not permit the morphological identification of the nigral neurones destroyed by kainic acid. But, in view of the existence of nigral non-DA neurones projecting to the thalamus, to the reticular formation or to the striatum¹²⁻¹⁶, lesion of these pathways could be the cause of the chronic effects of intranigral kainic acid.

The existence of nigral non-DA neurones controlling turning behaviour in a manner opposite to the nigrostriatal DA-system can provide an explanation for some turning responses obtained from the nigra which are not readily explained by an action on the nigrostriatal DA-system. For example, unilateral intranigral administration of ethanolamine-O-sulphate, which produces an accumulation of endogenous GABA by blocking GABA-transaminase, results in spontaneous contralateral turning^{17,18}. This syndrome resembles the chronic one produced by intranigral kainic acid and therefore might be due to a GABA-mediated depression of those nigral neurones destroyed by kainic acid.

G. DI CHIARA
M. OLIANAS
M. DEL FIACCO
P. F. SPANO
A. TAGLIAMONTE

Institutes of Pharmacology and of Anatomy,
University of Cagliari,
01900 Cagliari, Italy

Received 13 April; accepted 23 June 1977.

¹ Ungerstedt, U. & Arbuthnott, G. W. *Brain Res.* 24, 485-493 (1970).

² Schwartz, W. J., Gunn, R. H., Sharp, F. R. & Everts, E. V. *Brain Res.* 105, 358-361 (1976).

³ Iwamoto, E. T., Loh, H. H. & Way, E. L. *Eur. J. Pharmac.* 37, 339-356 (1976).

⁴ Costall, B., Marsden, C. D., Naylor, R. J. & Pycock, C. J. *Brain Res.* 118, 87-113 (1976).

⁵ Coyle, J. T. & Schwarcz, R. *Nature* 263, 224-246 (1976).

⁶ McGeer, E. G. & McGeer, P. L. *Nature* 263, 517-519 (1976).

⁷ Pellegrino, L. J. & Cushman, A. J. *A Stereotaxic Atlas of the Rat Brain* (Meredith, New York, 1971).

⁸ Kim, G. S., Hassler, R. & Okada, Y. *Expl Brain Res.* 14, 95-104 (1971).

⁹ McGeer, P. L., McGeer, E. G., Wada, J. A. & Jung, E. *Brain Res.* 32, 425-431 (1971).

¹⁰ Ribak, C. E., Vaughn, J. E., Saito, K., Barber, R. & Roberts, E. *Brain Res.* 116, 287-298 (1976).

¹¹ Buu, N. T. & Van Gelder, N. M. *Gen. Pharmac.* 7, 6-14 (1976).

¹² Feltz, P. & De Champlain, J. *Brain Res.* 43, 595-600 (1972).

¹³ Hattori, T., Fibiger, H. C., McGeer, P. L. & Maler, L. *Expl Neurol.* 41, 599-611 (1973).

¹⁴ Rinvik, E., Grofova, I. & Ottersen, O. P. *Brain Res.* 112, 388-394 (1976).

¹⁵ Faull, R. L. M. & Carman, J. B. *J. comp. Neurol.* 132, 73-92 (1968).

¹⁶ Carpenter, M. B. *Adv. Neurol.* (eds Eldridge, R. & Fahs, S.) (Raven, New York, 1976).

¹⁷ Dray, A., Fowler, L. J., Oakley, N. R., Simmonds, M. A. & Tanner, T. *Br. J. Pharmac.* 55, 288P (1975).

¹⁸ Koob, G., Del Fiacco, M. & Iversen, S. D. *Proc. Br. pharmac. Soc.*, abs. C.60 (1976).

Enkephalin receptors on dopaminergic neurones in rat striatum

THE idea that morphine and other narcotic analgesics as well as opioid peptides affect the activity of nigrostriatal dopaminergic neurones is well supported. For instance these substances elicit changes in motor activity similar to those observed after blockade of dopamine (DA) receptors by neuroleptics, they increase DA turnover in striatum and several typical symptoms of the abstinence syndrome they provoke involve dopaminergic systems (see refs 1-3 for reviews). In agreement with these observations, a high density of 'opiate receptors'⁴ and of enkephalin binding sites^{5,6}, a high content in enkephalins⁷ and a dense network of enkephalin-containing neurones⁸ are found in the striatum. All these observations suggested the possible connection between alleged enkephalinergic neurones and the dopaminergic fibres ending in the striatum. We report here that both the regional distribution of high affinity (morphine-displaceable) binding sites for leucine-enkephalin (Leu-enkephalin) in striatum and their decreased number after chemical or mechanical interruption of the nigrostriatal bundle support the assumption that enkephalinergic neurones terminate presynaptically on dopaminergic nerve terminals in the striatum.

³H-Leu-enkephalin binds to a particulate fraction from rat striatum and the saturation kinetics at equilibrium evidence two distinct sites; the first one, exhibiting a high affinity (K_d around 3 nM) and recognised by morphine or naloxone, has been identified as the 'opiate receptor' whereas the nature and possible function of the second one is not yet established^{9,10}. We have determined the number of binding sites for ³H-Leu-enkephalin displaceable by morphine in successive frontal sections of rat striatum (Fig. 1). The density of these sites decreases regularly from the head to the tail of the striatum, with a twofold variation between the first and the last dissected area. The topographical variation of DA content (Fig. 1), in agreement with the report of Tassin *et al.*¹¹, grossly parallels that of the sites of ³H-Leu-enkephalin binding displaceable by morphine. Since

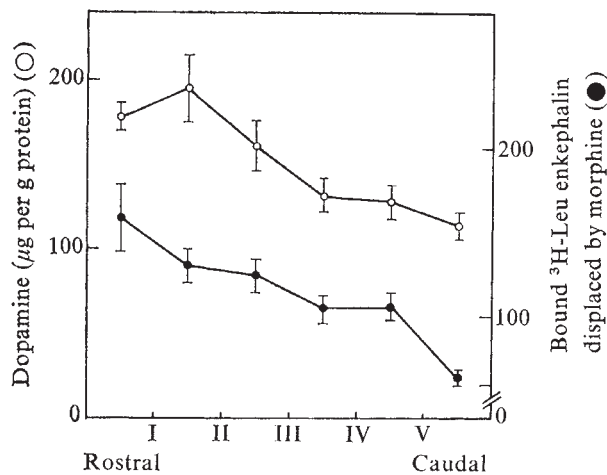


Fig. 1 Distribution of ³H-Leu-enkephalin binding sites displaceable by morphine and DA content in frontal sections of rat striatum. The rat striatum was dissected according to Glowinski and Iversen⁹, applied on a cold plate and divided into six serial slices. The first section was performed at the level of the anterior commissure (arrow) and therefore slice I included a portion of n. accumbens in addition to striatal tissue; the remaining tissue was then divided into five parallel slices of equal thickness. The good reproducibility of the dissection procedure was indicated by a variation of less than 6% between the protein content of homologous slices in different experiments. Homologous slices from four animals were pooled together and homogenised in 20 volumes of cold 50 mM Tris buffer, pH 7.4. An aliquot of the homogenate was quickly removed and deproteinised with HClO₄ for DA assay¹⁰. Binding of ³H-Leu-enkephalin^{5,6} was measured on a particulate fraction of the homogenate obtained by two successive centrifugations (180g for 5 min, then 10,000g for 20 min). 15-min incubations at 30 °C in the presence of 20 µM bacitracin, and 12 nM ³H-Leu-enkephalin (45.6 Ci mmol⁻¹, Radiochemical Centre, Amersham) alone or in the presence of 5 µM morphine. Bound ³H-Leu-enkephalin is expressed as fmol per mg of protein and represents the binding displaceable by morphine. Means ± s.e.m. from five separate experiments with triplicate assays for ³H-Leu-enkephalin binding and duplicate DA estimations. ○ Dopamine content. ●, ³H-Leu-enkephalin binding sites.

the variation in DA content reflects the heterogeneous distribution of DA terminals, our results suggest that enkephalin receptors might be topographically associated with dopaminergic synapses.

The presence of enkephalin receptors on DA neurones themselves was investigated by evaluating the effects of selective lesions of the nigrostriatal neurones on ³H-Leu-enkephalin binding. Degeneration of DA terminals was elicited either chemically by 6-hydroxydopamine (6-OHDA) or by electrocoagulation of the pars compacta of substantia nigra and neurochemically assessed by the reduction in 'dopa' decarboxylase activity (Table 1). Such lesions resulted after 6–22 d in significant decreases in ³H-Leu-enkephalin binding displaceable by morphine. The similar decrease in ³H-naloxone binding following the chemical lesions confirms that the high affinity binding sites of ³H-Leu-enkephalin represent the 'opiate receptors'. To confirm that the observed changes were indeed related to the degeneration of DA neurones, we have more selectively destroyed the nigrostriatal DA system by careful unilateral micro-injections of 6-OHDA into the substantia nigra in the conditions of Agid *et al.*¹¹. Two weeks later the decrease in ³H-Leu-enkephalin binding displaceable by morphine observed in the striatum of the lesioned side was by 27 ± 3% (means ± s.e.m. from nine animals) whereas the high affinity uptakes of ³H-choline and ³H-5-hydroxytryptamine¹³ in striatal synaptosomes from the same animals were not significantly modified. In addition, we have observed significant decreases in ³H-Leu-enkephalin and ³H-naloxone binding (by 30–40%) in rats with the nigrostriatal bundle sectioned at the hypothalamic level 10–30 d before (in preparation).

The decrease in ³H-Leu-enkephalin binding following electrocoagulation of the substantia nigra probably reflects a diminished number of receptor sites since the decrease affects the maximal binding on the high affinity sites without significant change in *K_d* (4.7 ± 0.8 nM on the control side compared with 4.2 ± 0.4 nM on the lesioned side) (Fig. 2).

Conceivably transynaptic degeneration may have a role in the observed changes but our observation, consistent with previous reports^{14,15}, that ³H-choline uptake is not affected indicates that striatal cholinergic neurones, on which DA neurones project, do not degenerate transynaptically. It might also be argued that the decreased number of Leu-enkephalin binding sites is due to the interruption of a neuronal system other than the dopaminergic one (or the serotonergic one which is unaffected after intranigral 6-OHDA). Such an hypothesis cannot be entirely ruled out but seems very unlikely because the decreased binding is elicited by three different types of lesions—electrolytic, mechanical and chemical—of which the common feature is to induce a degeneration of the DA terminals in the striatum.

Hence both the regional distribution of the high affinity Leu-enkephalin binding sites within the striatum and the decreased number of these sites after lesions might be interpreted on the assumption that enkephalinergic (opiate) receptors are localised on dopaminergic terminals. Assuming that the high affinity Leu-enkephalin binding sites represent postsynaptic receptors for the alleged enkephalinergic neurones, our findings would suggest that these neurones project presynaptically on to dopaminergic nerve terminals. This idea is strengthened by the immunohistochemical demonstration of Leu-enkephalin containing nerve

Table 1 Effects of substantia nigra lesions and intraventricular injections of 6-OHDA on binding of ³H-Leu-enkephalin or ³H-naloxone in rat striatum

Lesions	³ H-Leu-enkephalin binding displaceable by morphine (fmol per mg protein)	³ H-Naloxone binding (fmol per mg protein)	Dopa decarboxylase activity (nmol per mg protein per h)
Chemical lesion by intraventricular 6-OHDA			
Control animals	162 ± 10	66 ± 3	0.31 ± 0.02
Lesioned animals	126 ± 11*	47 ± 2†	0.20 ± 0.02‡
Variation	–22%	–28%	–33%
Electrocoagulation of the substantia nigra			
Control side	175 ± 11		0.30 ± 0.02
Lesioned side	134 ± 8*		0.19 ± 0.02‡
Variation	–23 ± 2%*		–37 ± 4%

For the chemical lesions the rats received intraventricularly 250 µg of 6-OHDA dissolved in 10 µl of saline containing 2% ascorbic acid. Controls received the same volume of vehicle. Unilateral electrocoagulations of the substantia nigra, pars compacta, were performed under chloral anaesthesia by passing a current of 80 mA for 5 s. Stereotaxic coordinates (*A* = +1.8 mm, *L* = 1.8 mm, *V* = –2.3 mm) refer to the König and Klippel atlas. Animals were killed 6–22 d after placement of lesions. Placement of electrocoagulations was checked histologically. ³H-Leu-enkephalin binding displaceable by morphine was estimated as described in Fig. 1 and typically represented about 1,000 d.p.m. per sample (0.15 mg protein) in controls; triplicate assays varied by less than 5%. Saturable ³H-naloxone binding was estimated on aliquots of the same particular fraction incubated with 3 nM ³H-naloxone (20 Ci mmol⁻¹, NEN Chemicals) alone or in the presence of 1 µM non-radioactive naloxone. DOPA decarboxylase activity was measured, on aliquots of the initial homogenate incubated for 40 min in the presence of 8 × 10⁻⁴ M ¹⁴C-carboxyl-labelled Dopa (Radiochemical Centre, Amersham). Means ± s.e.m. of five to eight separate experiments, on pools from one to three animals each.

**P* < 0.05

†*P* < 0.01

‡*P* < 0.005.

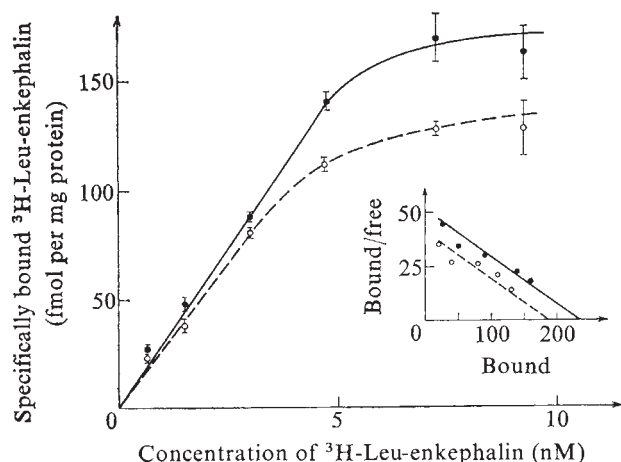


Fig. 2 High affinity binding of ³H-Leu-enkephalin to striatal membranes after unilateral electrocoagulation of the substantia nigra, pars compacta. Rats killed 10 d after placement of the lesions, as described in Table 1. Specifically bound ³H-Leu-enkephalin refers to the binding displaceable by 50 μ M non-radioactive Leu-enkephalin. Means $\pm$ s.e.m. from triplicate determinations on pooled striata from four animals. Insert: Scatchard plots of the same data. ●, Control side; ○, lesioned side.

terminals in rat striatum⁸, by the presence of the peptide in synaptosomal fractions from this region^{7,16} and finally, by the effect of morphine and opioid peptides on the release of DA from striatal slices^{17,18}.

The limited decreases in Leu-enkephalin receptors following our lesions indicate that only a fraction of these receptors in striatum are present on DA neurones. Even after sections of the nigrostriatal bundle at the hypothalamic level, resulting in an extensive depletion (>80%) of striatal DA the decrease in ³H-Leu-enkephalin binding was only 30–40%. This suggests that about one-third of enkephalin receptors in striatum are present on dopaminergic nerve terminals and that non-dopaminergic cells in the striatum might also receive enkephalinergic inputs.

From observations that morphine and endorphins decelerate the K⁺-induced release of DA slices of rat striatum^{17,18}, it might be inferred that the effect of enkephalin receptors on dopaminergic neurones could be to modulate DA release in an inhibitory fashion. This would account for the symptoms of a decreased dopaminergic neurotransmission, such as catalepsy, following the administration of morphine or endorphins to rats¹⁹; the paradoxical increase in DA synthesis might be only a secondary effect like that observed after various treatments resulting in a diminished release of the catecholamine^{20,21}.

There is already evidence that opiate receptors are also localised presynaptically on sensory fibres of the vagus nerve and the spinal cord²². In addition morphine and endorphins reduce the release not only of DA but also of other cerebral neurotransmitters like acetylcholine or nor-adrenaline³. Taken together with our data, these observations suggest that a major function of enkephalins could be to mediate presynaptic inhibitions on a variety of neuronal systems in the central nervous system.

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H. POLLARD
C. LLORENS-CORTES
J. C. SCHWARTZ

Unité de Neurobiologie (U.109),
Centre Paul Broca de l'INSERM,
2ter, rue d'Alésia,
75014 Paris, France

Received 17 March; accepted 1 July 1977.

- 1 Lal, H. *Life Sci.* **17**, 483–496 (1975).
- 2 Kuschinsky, K. *Arzneimittel-Forsch.* **26**, 563–567 (1976).
- 3 Eidelberg, E. *Prog. Neurobiol.* **6**, 81–102 (Pergamon, Oxford, 1976).
- 4 Kuhar, M. J., Pert, C. B. & Snyder, S. H. *Nature* **245**, 447–451 (1973).
- 5 Audigier, Y. *et al. C. r. heb. Séanc. Acad. Sci., Paris* **284**, 73–76 (1977).
- 6 Audigier, Y., Malfroy-Camine, B. & Schwartz, J. C. *Eur. J. Pharmac.* **41**, 247–248 (1977).
- 7 Smith, T. W., Hughes, J., Kosterlitz, H. W. & Sosa, R. P. in *Opiates and Endogenous Opioid Peptides* (ed. Kosterlitz, H. W.) 57–62 (Elsevier-North-Holland, Amsterdam, 1976).
- 8 Elde, R., Hokfelt, T., Johansson, O. & Terenius, L. *Neuroscience* **1**, 349–351 (1976).
- 9 Glowinski, J. & Iversen, L. L. *J. Neurochem.* **13**, 655–669 (1966).
- 10 Lavery, R. & Taylor, K. M. *Analyt. Biochem.* **22**, 269 (1968).
- 11 Tassin, J. P., Cheramy, A., Blanc, G., Thierry, A. M. & Glowinski, J. *Brain Res.* **58**, 291–301 (1976).
- 12 Agid, Y., Javoy, F., Glowinski, J., Bouvet, D. & Sotelo, C. *Brain Res.* **58**, 291–301 (1977).
- 13 Kuhar, M. J. *Life Sci.* **13**, 1623–1634 (1973).
- 14 Grewal, D. S., Fibiger, H. C. & McGeer, E. G. *Brain Res.* **73**, 372–375 (1974).
- 15 Agid, Y., Guyenet, P., Glowinski, J., Beaujouan, C. & Javoy, F. *Brain Res.* **86**, 488–492 (1975).
- 16 Simantov, R. & Snyder, S. H. in *Opiates and Endogenous Opioid Peptides* (ed. Kosterlitz, H. W.) 41–48 (Elsevier-North-Holland, Amsterdam, 1976).
- 17 Celsens, B. & Kuschinsky, K. *Naunyn-Schmiedberg's Archs Pharmac.* **284**, 159–164 (1974).
- 18 Loh, H. H., Brase, D. A., Sampath-Khanna, S., Mar, J. B. & Way, E. L. *Nature* **264**, 567–568 (1976).
- 19 Bloom, F., Segal, D., Ling, N. & Guillemin, R. *Science* **184**, 630–632 (1976).
- 20 Carlsson, A. in *Pre- and Post-synaptic Receptors, Annual ACNP Meeting Puerto Rico* (eds Usdin, E. & Bunney, W. E., Jr) (Marcel Dekker, New York, 1975).
- 21 Roth, R. H. *Eur. J. Pharmac.* **15**, 52–59 (1971).
- 22 Snyder, S. H. & Simantov, R. *J. Neurochem.* **28**, 13–20 (1977).

Blowfly ovipositor receptor neurone sensitive to monovalent cation concentration

THE ability of insects to taste salts with sense organs on their legs and mouthparts is well documented. Studies of taste hairs on the tarsi and labella of the black blowfly *Phormia regina* and the bluebottle *Calliphora erythrocephala* have shown that these contain several sensory cells, one of which is a 'salt' or 'type I' primary receptor neurone responding positively to the concentration of monovalent cations^{1–6}. I report here some of the receptor characteristics of monovalent cation sensitive neurones found in hair sensilla on the egg-laying apparatus of the sheep blowfly.

Sensory hairs with terminal pores have been identified on the ovipositors of the Australian sheep blowfly *Lucilia cuprina* and the migratory locust *Locusta migratoria*^{7–9}. As there is much behavioural evidence to suggest that insects taste their oviposition medium with their ovipositors^{9–12}, it is likely that the newly described sensilla are the gustatory receptors that mediate such behavioural reactions. In *L. cuprina* these sensilla have been shown, by electrophysiological recording, to contain at least three neurones which respond to stimulation with various chemical solutions⁷. Some gustatory sensilla of caterpillars respond to olfactory stimuli¹³, however, and as the ovipositor of the blowfly *P. regina* is known to make directed movements in response to olfactory stimuli¹⁴, it is of interest to know more about the receptor properties of blowfly ovipositor sensilla.

Gravid female *L. cuprina* from our culture of the GL strain were used. Conventional stimulating and recording equipment was used following the procedure of Hodgson *et al.*¹⁵. The glass micropipette containing the stimulating solution and recording electrode was placed over the tips of each of the 1g–5g sensilla⁷ in turn. All of the g sensilla were found to contain a neurone whose rate of firing increased with increasing concentration of applied sodium chloride solutions (Fig. 1 shows a typical result).

Sensillum 1g was the easiest to record from and all results given here were obtained from 1g sensilla. During this work recordings have been made from more than eighty 1g sensilla. The oscilloscope time-base was triggered by the contact artefact, a dual sweep displaying one trace at 10 times the speed of the other, the high speed trace sampling the initial part of the slower trace. This enabled analysis both of the initial rate of firing and also of the adaptation rate. The fast trace was also useful for inspection of the action potential shapes (Figs 1, 2).

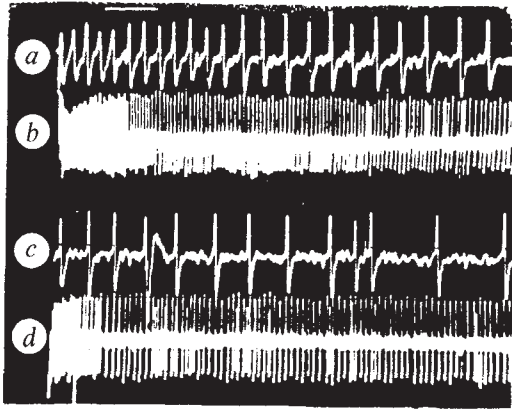


Fig. 1 Effect of NaCl concentration on the initial discharge frequency of the monovalent cation receptor neurone in the 1g sensillum on the ovipositor of *Lucilia cuprina*. a, b, 1.5 M NaCl; c, d, 0.15 M NaCl. Traces start at moment of stimulus application. Scale line: 20 ms in (a) and (c); 0.2 s in (b) and (d).

Action potentials of three different amplitudes were recorded from the g sensilla on stimulation with salt solutions: small- (1), medium- (2), and large- (3) sized action potentials (Fig. 2). When stimulated with monovalent salts, the labellar taste hairs of *Calliphora erythrocephala* also produce three sizes of impulses which have been tentatively identified as cation, anion and water receptors respectively¹⁶. In the 1g receptors of *L. cuprina* action potentials from cell 2 are always obtained in response to stimulation with solutions of monovalent cation chlorides, the smaller cell 1 action potentials sometimes accompany cell 2 responses, generally starting to discharge after prolonged stimulation, while the larger cell 3 action potentials are a more rare accompaniment. Responsiveness of the g sensilla varied from fly to fly being influenced by the age and nutritional status of each individual. By comparing the relative stimulating power of a series of cations and anions on single 1g sensilla, replicable results were achieved. Receptor cell 2 was found to be almost equally responsive to 0.15 M solutions of NaCl, NaBr and NaI but much less responsive to solutions of NaF (Fig. 3). The rate of firing of this neurone is largely dependent on the concentration of monovalent cation present in the stimulating solution and, apart from NaF, is not much effected by the anions tested, which included NO_3^- and SO_4^{2-} . A range of monovalent cation chlorides were almost equally stimulatory, though the Li^+ response had a faster adaptation rate than either Na^+ or K^+ (Fig. 3).

In contrast to its concentration-dependent responses to monovalent cations, neurone 2 is much less responsive to

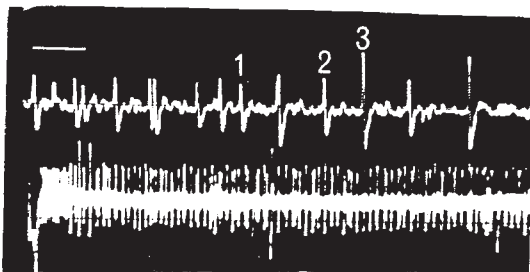


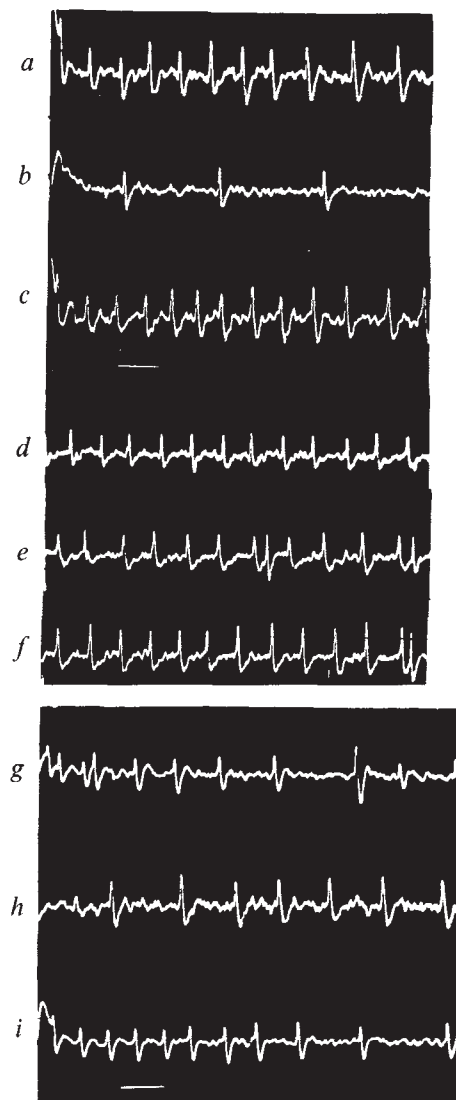
Fig. 2 Three-unit discharge, infrequently recorded from sensillum 1g. Stimulus is 0.15 M NaCl. The three different amplitudes of action potentials are indicated by the numerals (1-3). An odd shaped spike occurs between 1 and 2, this is thought to be due to the almost simultaneous firing of two units. Scale line: 20 ms in upper trace and 0.2 s in lower trace.

the divalent cations Ca^{2+} , Mg^{2+} and Sr^{2+} and is totally unresponsive to a range of concentrations of choline chloride. Divalent cations at a concentration of 0.15 M were sometimes stimulatory (Fig. 4) but a 10-fold increase in concentration did not increase the response as it always does with monovalent cations. More details of the responses of 1g sensilla to divalent cations will be published elsewhere.

When ranges of NaCl and KCl solutions were tested a logarithmic relationship was found, with the type 2 cell approximately doubling its initial rate of firing for each 10-fold increase in molar concentration of Na^+ or K^+ ions. Threshold for cell 2 was as low as 0.001 M NaCl in several preparations, which is 100 times lower than was found in the salt receptors of the labellar hairs of *P. regina*¹⁷. This may reflect the need of the sheep blowfly to identify this salt with its ovipositor receptors when attempting to lay eggs on diluted serous exudates in the wet wool of sheep, sodium being the main monovalent cation of serum.

The cell 2 receptor neurone in the 1g sensillum of the ovipositor of *L. cuprina* is specialised for detection of monovalent cation concentrations. This neurone is very similar to the 'salt' or 'type I' receptor neurones that are so well known from the taste hairs of the blowfly labellum and

Fig. 3 Single-unit responses from two 1g sensilla in response to 0.15 M salt solutions. Traces a-f: responses to sodium halides; chloride acting as the control; traces g-i: responses to monovalent cation chlorides. a, NaCl; b, NaF; c, NaCl; d, NaBr; e, NaI; f, NaCl; g, NaCl; h, KCl; i, LiCl. Scale lines, 20 ms.



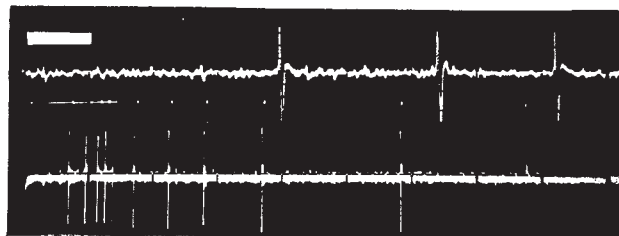


Fig. 4 Single-unit responses from a 1g sensillum to 0.15 M CaCl_2 solution. Scale line: 20 ms upper, 0.2 s lower trace.

tarsus¹⁻⁸. This work now adds the ovipositor as the third position in which such receptor neurones are found. The behavioural role of monovalent cation receptors on the sheep blowfly ovipositor is likely to be in the selection of suitable egg-laying sites. When blowflies lay eggs on sheep or on artificial media, probing of the substrate with the ovipositor precedes the deposition of eggs. Locusts and parasitic wasps only lay eggs after the ionic composition of the substrate has been monitored by probing with the ovipositor^{9,11}. It is interesting to speculate that alteration of the ion composition on the integument of sheep may influence the egg-laying behaviour of sheep blowflies.

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M. J. RICE

Department of Entomology,
University of Queensland,
St Lucia, Queensland 4067, Australia

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- Hodgson, E. S. in *The Physiology of the Insects* 2 (ed. Rockstein, M.) ch. 4 (Academic, New York, 1974).
- Dethier, V. G. *The Hungry Fly* (Harvard University Press, 1976).
- Den Otter, C. J. *Net. J. Zool.* 21, 464-489 (1971).
- Rees, C. J. *J. Insect Physiol.* 14, 1331-1364 (1968).
- Steinhardt, R. A. *Am. Zool.* 5, 651 (1965).
- Evans, D. R. & Mellon, E. J. *gen. Physiol.* 45, 651-661 (1962).
- Rice, M. J. *Aust. J. Zool.* 24, 353-360 (1976).
- Rice, M. J. & McRae, T. M. *J. Aust. ent. Soc.* 15, 364 (1977).
- Dethier, V. G. *J. Exp. Zool.* 105, 199-207 (1947).
- Edwards, R. L. *Behaviour* 7, 88-112 (1954).
- Woodrow, D. F. *Anim. Behav.* 13, 348-356 (1965).
- Ganesalingam, V. K. *Entomol. exp. Appl.* 17, 36-44 (1974).
- Stadler, E. & Hanson, F. E. *J. comp. Physiol.* 104, 97-102 (1975).
- Barton Browne, L. J. *Insect Physiol.* 5, 16-22 (1960).
- Hodgson, E. S., Lettvin, J. Y. & Roeder, K. D. *Science* 122, 417-418 (1955).
- Den Otter, C. J. & Van Der Poel, A. M. *Nature* 206, 31-32 (1966).
- Gillary, H. L. *J. gen. Physiol.* 50, 359-368 (1966).

Non-cholinergic excitatory transmission to intestinal smooth muscle cells

CONTRACTILE responses of the chicken rectum to stimulation of Remak's nerve and intramural nerves are virtually unaffected by drugs affecting the cholinergic or adrenergic system, suggesting that the remaining response is mediated through excitation of nerves other than cholinergic or adrenergic nerves¹⁻³. Using the sucrose-gap method we found excitatory junction potentials assumed to be elicited by stimulation of excitatory nerves whose transmitter was unknown⁴. In an attempt to confirm and extend these observations we have studied an electrical change in the smooth muscle membrane, recorded intracellularly, in junctional transmission from excitatory nerves.

The rectum was isolated together with Remak's nerve running parallel to the longitudinal axis of the organ and with its several branches extending to the intestinal wall. This was sectioned lengthwise and mounted in a chamber provided with electrodes which allow Remak's nerve and intramural nerves to be stimulated separately. The membrane potential of smooth muscle cells was measured using conventional glass microelectrodes filled with 3 M KCl and having a resistance of 60-100 M Ω .

Stimulation of the anal cut end of Remak's nerve with single square pulses (0.5 ms duration, at a supramaximal intensity) gave action potentials in cells along the entire surface of the rectum in most preparations. In the majority of cells, action potentials arose from slow depolarisations of the membrane potential which range up to 15 mV from the resting membrane potential (-48.6 ± 2.6 mV, $n=80$) and are interpreted to be excitatory junction potentials (EJPs) elicited by a transmitter released from nerve endings (Fig. 1a). In some cells, they fired during the falling phase of EJPs or without any preceding changes in the membrane potential.

The amplitude of the EJPs varied both from preparation to preparation and from cell to cell in any one preparation. Their amplitude also varied with the stimulus intensity. It was possible to evoke subthreshold EJPs by decreasing the stimulus voltage. At any stimulus intensity, stimulation of the oral cut end of Remak's nerve was much less effective in producing the electrical response. These results suggest that the smooth muscle cells in the rectum receive a multiple innervation from the excitatory nerves in varied

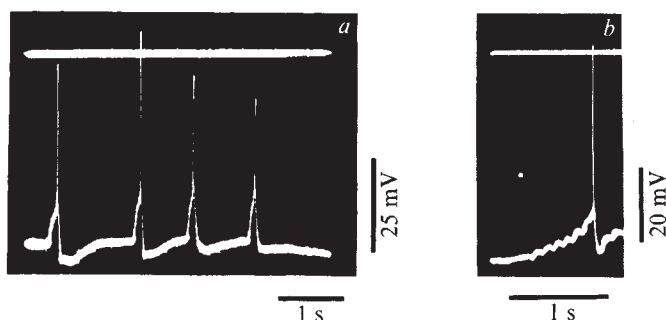


Fig. 1 Intracellular records from smooth muscle cells in the chicken rectum during stimulation with pulses of 0.5 ms duration of Remak's nerve. a, Action potentials arose from slow depolarisations of the membrane potential (interpreted to be excitatory junction potentials); b, summation of excitatory junction potentials, evoked by repetitive stimulation at 10 Hz, over threshold for initiation of the action potential in another cell.

densities and that the excitatory nerve fibres originate mainly in the ascending fibres of Remak's nerve. In all preparations in which cells giving only an EJP were observed, a train of pulses at frequencies less than 10 Hz facilitated EJPs until the depolarisation reached a level to initiate the action potential (Fig. 1b). The observed latency before onset of EJPs was 40-65 ms with a mean of 55.0 ± 2.9 ms ($\pm$ s.e.). Latency of EJPs was not consistent in cells along the longitudinal axis of the preparation, but it did not significantly vary along the transverse axis; as the microelectrode was moved farther away from the stimulating site, the latency prolonged. The increased latency was up to 20 ms in three preparations after shifting the recording site 20 mm away. EJPs took from 55-100 ms to attain the maximum height after the onset and lasted 600-850 ms.

Field stimulation of intramural nerves (0.1 ms duration) also gave an EJP with latency shorter than those of EJPs elicited by Remak's nerve stimulation. The latencies estimated in EJPs recorded from 30 cells in 8 different preparations varied from 5 to 15 ms with a mean of 9.2 ± 1.2 ms ($\pm$ s.e.). EJPs in response to Remak's nerve stimulation, but not those to field stimulation, were blocked by hexamethonium (10^{-4} g ml⁻¹ or less). Thus, it was apparent that there was ganglionic transmission mediated via acetylcholine receptors of the nicotinic type in the motor pathway from Remak's nerve to the rectum and that the delay of EJPs after the nerve stimulation is largely due to synaptic transmission at the ganglion. Neither atropine nor hyoscine in

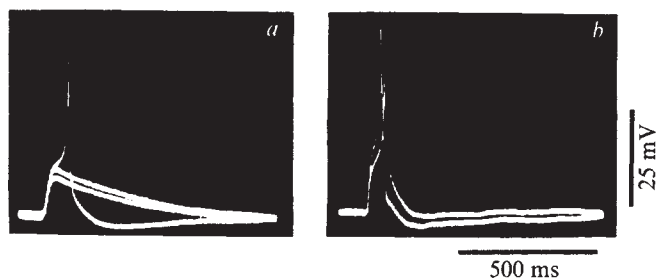


Fig. 2 Effect of atropine on excitatory junction potentials evoked by field stimulation with pulses of 0.1 ms duration at 0.8 Hz. *a*, Control; *b*, 5 min after addition of atropine, 5×10^{-7} l $^{-1}$, to the bathing solution. Note that the excitatory junction potentials were appreciably potentiated by atropine.

concentrations up to 10^{-6} g ml $^{-1}$ inhibited EJPs evoked by stimulation of the extrinsic and intrinsic nerves and instead these drugs invariably potentiated the EJPs. This effect is illustrated in Fig. 2, where the EJPs are evoked by field stimulation with single pulses at an interval of 1,250 ms before and after application of atropine (5×10^{-7} g ml $^{-1}$). In these stimulus conditions, facilitation of EJPs was seen in this cell and the third EJP reached the threshold membrane potential to trigger the action potential. In contrast, depolarisation of the first EJP was sufficient for firing the action potential in the presence of atropine. Physostigmine at between 10^{-9} and 10^{-7} g ml $^{-1}$ did reduce the EJPs without change in the membrane potential. Drugs affecting adrenergic functions had no effect on the EJPs. Tetrodotoxin 10^{-7} g ml $^{-1}$ abolished the electrical responses to field stimulation as well as Remak's nerve stimulation.

It is very likely that neurones directly involved in generation of the EJPs are neither cholinergic nor adrenergic. It is possible that there is a muscarinic mechanism by which the motor transmission to the smooth muscle cells is inhibited.

T. TAKEWAKI
O. OHASHI

Department of Pharmacology,
Faculty of Agriculture,
Gifu University,
Kakumigahara, Gifu 504, Japan

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¹ Takewaki, T., Ohashi, H. & Okada, T. *Jap. J. Pharmac.* 27, 105 (1977).

² Bartlet, A. L. & Hassan, T. Q. *J. exp. Physiol.* 56, 178 (1971).

³ Bartlet, A. L. *Br. J. Pharmac.* 51, 549 (1974).

⁴ Ohashi, H., Naito, K., Takewaki, T. & Okada, T. *Jap. J. Pharmac.* (in the press).

Calcium transients in frog slow muscle fibres

MANY muscles in the frog contain slow as well as twitch muscle fibres, which differ in their innervation, electrical and contractile properties and fine structure¹. Slow fibres give graded slow contractions with nerve stimulation, and can maintain a prolonged contracture when depolarised. There is evidence that contractile activation in slow fibres is mediated by a rise in myoplasmic calcium concentration², but it is not clear whether this calcium originates from the sarcoplasmic reticulum, as in twitch fibres, or enters the fibre from the external solution^{1,3-5}. We have used the calcium indicator dye arsenazo III (refs 6-9) to follow changes in intracellular free calcium concentration occurring during depolarisation of slow fibres, and find that the membrane potential dependence of these calcium transients in slow fibres is very similar to that observed in twitch fibres⁹. The time courses of the calcium transients in slow fibres are, however, very much slower than in twitch

fibres^{9,10}, and may be a major factor in determining the time courses of tension development and relaxation.

Experiments were performed on the pyriformis muscle of *Rana temporaria*¹¹, using a bathing solution of composition (mM): NaCl, 120; KCl, 2; CaCl₂, 12; pH, 7. Intracellular calcium changes with arsenazo III were measured as previously described for twitch fibres^{8,9}. Briefly, by cutting away the lower part, the muscle was reduced to a thin sheet of fibres which was stretched until contraction was practically abolished. Fibres were impaled with two electrodes, about 150 μ m apart, for voltage recording and dye injection. Slow fibres were identified by their inability to generate action potentials and their slow electronic time constant when tested with current pulses applied through the dye pipette. The membrane potential was held at -100 mV by passing a hyperpolarising current through the dye pipette, and this served also to slowly inject arsenazo III into the fibre. Changes in light absorption of the dye were monitored by focusing light from a 50-W bulb on to a spot of about 80 μ m diameter between the pipettes, and measuring the transmitted light at wavelengths of 532 and 602 nm using two photomultipliers and interference filters. The light absorbance of arsenazo III changes in opposite directions at these wavelengths in the presence of calcium, and by subtracting the signals from the two photomultipliers a record is obtained which gives a quantitative measure of changes in free calcium concentration, but is little affected by movement artefacts^{8,9}.

Changes in intracellular free calcium concentration produced by depolarising pulses are shown in Fig. 1. The size of the calcium transient is a graded function of membrane potential, and the rates of increase and decay of calcium concentration are much slower than in twitch fibres^{9,10}. The decay time constant measured in six fibres varied between 0.6 and 4 s, and the maximum rate of increase in free calcium concentration in 10 fibres was 0.045μ M ms $^{-1}$.

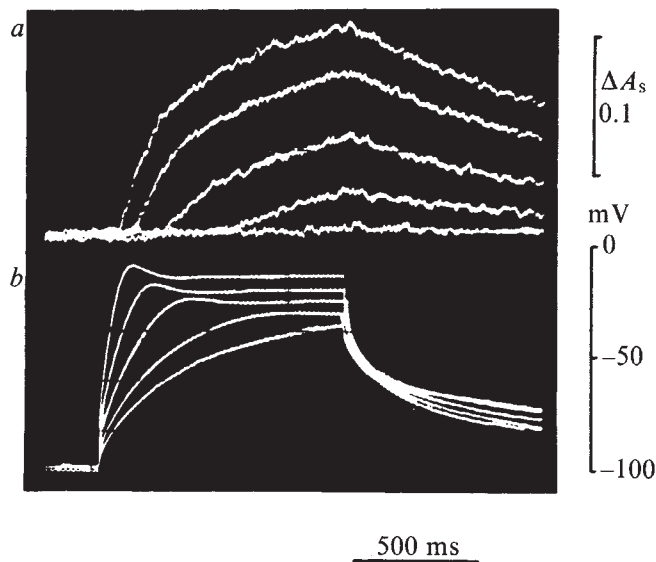


Fig. 1 Light absorbance records from a fibre injected with arsenazo III, showing responses to depolarising pulses of different amplitudes. *a*, Light absorbance changes at the wavelength pair 532-602 nm. *b*, Membrane potential. The light absorbance traces were recorded through a low-pass filter of time constant 10 ms, and the calibration (ΔA_s) gives the change in absorbance as a fraction of the total absorbance of the injected dye at a wavelength of 532 nm. An upward deflection indicates an increase in free calcium concentration, and ΔA_s of 0.1 is equivalent to an increase in free calcium of 2μ M (ref. 9). Five superimposed sweeps are shown. Stimuli were constant current pulses of 1 s duration and varying amplitudes. Temperature 7°C.

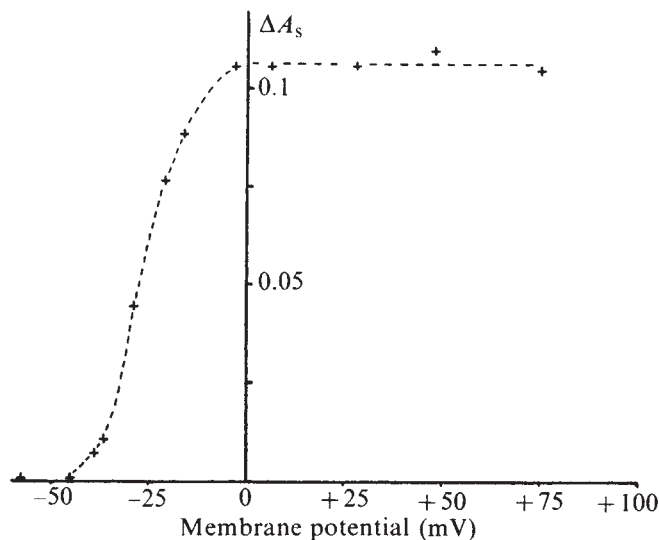


Fig. 2 Relationship between calcium response and membrane potential recorded in one fibre using depolarising pulses of 100 ms duration. Abscissa: membrane potential during pulse. Ordinate peak change in standardised light absorbance (ΔA_s) at the wavelength pair 532–602 nm. The fibre was voltage clamped using the dye pipette as a current-passing electrode, and the membrane potential was held at -90 mV between pulses. Temperature, 7°C .

± 0.025 (mean $\pm$ s.d.), when depolarised to a potential above that required to obtain a maximal response (compare Fig. 2). Corresponding values for twitch fibres are 50 ms and $0.95 \mu\text{M ms}^{-1}$ (ref. 9). The rise in calcium concentration during depolarisation shows two phases: an initial rapid increase, followed by a transition to a much slower rate of rise. This is probably a property of the calcium release mechanism, and is unlikely to be due to any nonlinearity in the recording system as the transition occurs at different calcium concentrations for different sized pulses, and the rate of calcium uptake is probably too slow to account for the transition.

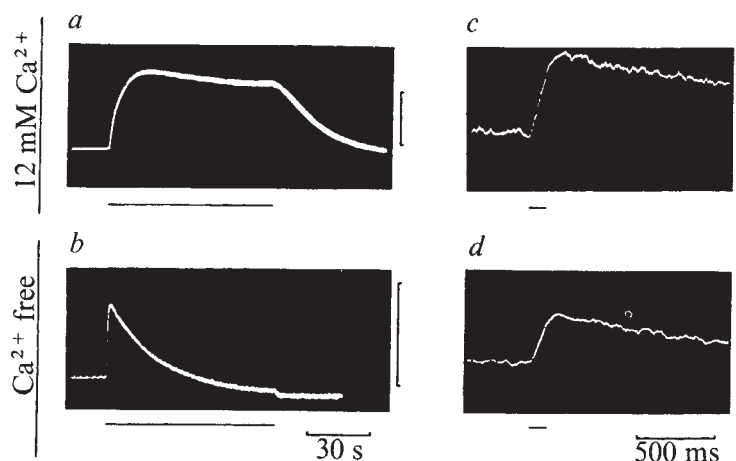
To examine the relationship between membrane potential and calcium release the membrane potential was clamped at the measuring spot, and the peak size of the calcium transient measured for different amplitudes of 100 ms depolarising pulses (Fig. 2). The calcium transient is a sigmoid function of membrane potential over a range of approximately -45 to 0 mV, and the relationship is very similar

to that found in twitch fibres⁹. With large depolarisations ($+75$ mV) the calcium response is not depressed, which suggests that the entry of external calcium is not important in the generation of the calcium transient, since the driving force for calcium entry would be reduced at this potential^{9,12}.

The role of external calcium was further studied by buffering the calcium concentration in the bathing solution at $<10^{-7}$ M with 1 mM EGTA and 5 mM MgCl_2 . We found no changes in the calcium transients produced by 100-ms depolarising pulses in this solution: mean response sizes produced by supramaximal depolarising pulses in 12 mM calcium and calcium-free solution were respectively $2.0 \mu\text{M} \pm 1.1$ ($n = 9$) and $1.4 \mu\text{M} \pm 0.4$ ($n = 4$), while the relationship between membrane potential and calcium release and uptake remained the same (Fig. 3c,d). During a prolonged depolarisation, however, the calcium response is considerably affected by removal of calcium from the bathing solution, and the response becomes transient, rather than maintained (Fig. 3a, b). This effect is well known from studies of contractile tension¹³, and might result from either an action of external calcium on the activating sites in the T-tubule membrane, or from the absence of a calcium influx which is required to maintain the intracellular calcium level. To test the importance of calcium influx during maintained contractures in 12 mM calcium, we gave a step depolarisation to $+80$ mV during prolonged depolarisations to $+20$ mV. This additional depolarisation should have reduced any calcium influx, but all experiments showed a small rise in intracellular calcium concentration, rather than a fall.

These experiments are in agreement with other work³ in demonstrating that an influx of external calcium is not directly involved in contractile activation in slow fibres, and the striking similarity in the relationships between membrane potential and calcium response for slow and twitch fibres supports the view that activation in slow fibres occurs by the same mechanisms as in twitch fibres^{4,5,13}. External calcium is, however, important in the maintenance of prolonged contractures, possibly by affecting the rate of inactivation of the activating sites within the T tubules^{13,14}. The slow rates of increase and decay of intracellular calcium in slow fibres may arise simply as a consequence of the relative scarcity of sarcoplasmic reticulum and triads^{15–17}, and are probably major factors in determining the rates of contraction and relaxation in slow muscle. Our values for the rate of decay of the calcium transient are in good agreement with measurements of tension relaxation^{1,5,18}, but the slow shortening velocity of the myofibrils² may also be an important factor in determining the rate of tension development.

Fig. 3 Effects of removal of external calcium on the calcium responses to prolonged (a and b) and short (c and d) depolarising pulses. In each record the fibre was depolarised from -100 mV to $+20$ mV during the times indicated by the bars, and all records were obtained from different fibres. Ringer solution containing 12 mM CaCl_2 (plus 120 mM NaCl and 2 mM KCl) was used in a and c, while in b and d the solution was (mM): NaCl , 120 ; KCl , 2 ; MgCl_2 , 5 ; EGTA, 1 ; pH 7 . Traces show changes in light absorbance at the wavelength pair 532–602 nm, and the calibration bars are $\Delta A_s = 0.5$ for a and b, and $\Delta A_s = 0.1$ for c and d. In b a small shift of the baseline occurred due to movement of the fibre. Temperature, 7.5°C .



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R. MILEDI
I. PARKER
G. SCHALOW*

Department of Biophysics,
University College London,
Gower Street, London WC1, UK

Received 9 May; accepted 28 June 1977.

*Present address: I Physiologisches Institut der Universität des Saarlandes, 665 Homburg/Saar, FRG.

- ¹ Lannergren, J. in *Basic Mechanisms of Ocular Motility and their Clinical Implications* (eds Lennérstrand, C. & Bach-y-Rita, P.) (Pergamon, Oxford, 1975).
- ² Costantin, L. L., Podolsky, R. J. & Tice, L. W. *J. Physiol., Lond.* **188**, 261–271 (1967).
- ³ Kirby, A. C. *Am. J. Physiol.* **219**, 1446–1450 (1970).
- ⁴ Nasledov, G. A., Mandelstam, J. E. & Radzjukewich, T. L. *Experientia* **28**, 1305–1306 (1972).
- ⁵ Gilly, W. F. & Hui, C. S. *Nature* **266**, 186–188 (1977).
- ⁶ Brown, J. E. *et al. Biophys. J.* **15**, 1155–1160 (1975).
- ⁷ Dipolo, R. *et al. J. gen. Physiol.* **67**, 433–467 (1976).
- ⁸ Miledi, R., Parker, I. & Schalow, G. *J. Physiol., Lond.* **269**, 11–13P (1977).
- ⁹ Miledi, R., Parker, I. & Schalow, G. *Proc. R. Soc. B* (in the press).
- ¹⁰ Taylor, S. R., Rudel, R. & Blinks, J. R. *Fedn Proc.* **34**, 1379–1381 (1975).
- ¹¹ Stefani, E. & Steinbach, A. B. *J. Physiol., Lond.* **203**, 383–401 (1969).
- ¹² Katz, B. & Miledi, R. *J. Physiol., Lond.* **192**, 407–436 (1967).
- ¹³ Lannergren, J. *Acta physiol. scand.* **69**, 362–372 (1967).
- ¹⁴ Chandler, W. K., Rakowski, R. F. & Schneider, M. F. *J. Physiol., Lond.* **254**, 285–316 (1976).
- ¹⁵ Page, S. G. *J. Cell Biol.* **26**, 477–497 (1965).
- ¹⁶ Franzini-Armstrong, C. *J. Cell Biol.* **56**, 120–128 (1973).
- ¹⁷ Bailey, C. H. & Peachey, L. D. *J. Cell Biol.* **67**, 15a (1975).
- ¹⁸ Kuffler, S. W. & Vaughan Williams, E. M. *J. Physiol., Lond.* **121**, 318–340 (1953).

Induction of experimental allergic neuritis with a peptide from myelin P₂ basic protein

EXPERIMENTAL allergic neuritis (EAN) is an autoimmune demyelinating disease of the peripheral nervous system (PNS) produced in animals by injection of homogenates of PNS tissue¹ or PNS myelin² emulsified with Freund's complete adjuvant. Although it has been produced in such species as rabbits¹, guinea pigs³, and mice³, the neuritogenic determinant has not been identified. In experimental allergic encephalomyelitis (EAE), the central nervous system (CNS) counterpart of EAN, the encephalitogen is the myelin basic protein⁴. PNS myelin has two basic proteins, P₁ and P₂⁵, and the latter has been suggested to be the neuritogen². In attempts to induce the disease with intact P₂ (refs 6–9), mild symptoms of EAN were occasionally produced, but these were inconsistent. P₂ was implicated as the neuritogen when circulating lymphocytes of animals with EAN induced by injection of whole PNS myelin¹⁰ were found to be sensitised to P₂ but not P₁. Physicochemical studies of P₂ have demonstrated a very stable β structure in aqueous solution¹¹. Because the conformation is likely to differ in solution and in intact myelin, it is likely that the neuritogenic determinant(s) are altered. We have, therefore, tested peptides derived from the protein in order to minimise alteration of any neuritogenic determinant by extraneous portions of the intact molecule. We have identified a

neuritogenic determinant within P₂—within a peptide representing the NH₂-terminal 21 amino acids of P₂.

P₂ (55 mg) was digested with cyanogen bromide (CNBr) (ref. 12) (in 3.0 ml 70% formic acid) and the resultant peptides were separated by chromatography on a column (1.5 × 69 cm) of Sephadex G-50. The column was equilibrated and eluted with 0.1 M acetic acid at 35 ml per h; 3.0 ml fractions were collected. Two main fragments were isolated and designated fragment I (fractions 10–35) and fragment II (fractions 38–43). Fragment II had a blocked NH₂-terminal and 21 amino acids. Fragment I comprised the bulk of the remaining molecule. The isolation and characterisation of the CNBr peptides will be described in detail elsewhere.

When tested for disease in rabbits, fragment II produced histological lesions characteristic of allergic neuritis—perivascular infiltration of mononuclear cells and demyelination (Table 1). The two rabbits receiving 300 μ g each of fragment II did not develop clinical symptoms. Two of the three receiving 500- μ g doses developed a general weakness and weight loss while the third developed marked clinical symptoms including ataxia and severe weakness of the hind quarters. The symptoms in this rabbit and the histological lesions in all the affected rabbits were similar to those observed when either whole PNS homogenates¹ or purified PNS myelin² is the inducing agent. The CNS remained essentially unchanged in these animals. Occasional lesions were observed in the spinal cord in the area of the root entry zones but these were interpreted as infiltration of cells responding to PNS myelin and overflowing into the adjacent spinal cord tissue.

Rabbit OR4 (Table 1) also had a few lesions along the ventral fissure of the lumbar cord. This animal had such an overwhelming inflammation and acute infiltration of cells throughout the PNS, especially in the dorsal root ganglia, that some spinal cord involvement extending beyond the area of the root entry zone was not surprising. Because this was the only area involving CNS tissue and was mild in comparison with the PNS involvement, it was interpreted as a nonspecific consequence of the cell-mediated response to the neuritogen, a phenomenon similar to that reported by Wisniewski and Bloom¹⁴.

When fragment I was injected into rabbits (1 mg per animal), some histological lesions were observed, suggesting the possibility of additional neuritogenic determinant(s) in the remainder of the molecule. This latter observation is being further investigated.

Thus it seems that P₂ contains at least one determinant for EAN in the rabbit. Apparently this neuritogenic determinant is inaccessible in the isolated intact protein, probably as a result of the non-native β structure. When removed from the bulk of the protein, fragment II must assume a conformation which approximates that found in the intact myelin.

Since P₁ can also cause disease⁶ the question of contamination of P₂ or fragment II with the encephalitogenic P₁ protein must be considered. In our hands, when rabbits are sensitised with P₂, no histological lesions are noted in either the CNS or PNS and only rarely are a few transitory clinical symptoms observed⁷. Thus, our P₂ preparation does not seem to be contaminated with P₁. It is even

Table 1 Induction of EAN* in rabbits by fragment II†

Animal no.	D (μ)	Clinical symptoms (day of onset)	Day of killing	Histology‡			
				Perivascular cuffing	PNS	Demyelination	PNS
				CNS		CNS	
R 30	300	—	27	0	+/-	0	+/-
R 31	300	—	27	0§	+	0	+
OR 4	500	Weight loss, ataxia, and hind quarter weakness (19)	20	+/-	++	+/-	+
OR 8	500	Weight loss and weakness (23)	28	0	+	0	+
OR 9	500	Weight loss and weakness (21)	28	0	+	0	+

*Antigen was dissolved in saline and emulsified with an equal volume of Freund's complete adjuvant (H37 ra) containing 1 mg ml⁻¹ *Mycobacterium tuberculosis*. Each rabbit received 0.1 ml in each of its four footpads (total 0.4 ml per animal).

†Fragment II, the NH₂-terminal 21 amino acid peptide from bovine peripheral nerve myelin P₂ basic protein.

‡The system used to grade the histology is similar to that of Alvord and Kies¹²; the signs used are: 0, no abnormality; +/-, a few small foci of perivenular leukocytes within neuraxis; ++, large and numerous foci of perivenular leukocytes with neuraxis.

§Two foci of a few mononuclear cells in the white matter of the lumbar cord were observed.

||Refers to entire neuraxis. There were also some ++ lesions in the ventral fissure of the lumbar spinal cord with subpial demyelination. These lesions occasionally extended into the deep white or grey matter and were also demyelinating.

more unlikely that the fragment II preparation isolated from P₂ is contaminated since it is processed further after CNBr cleavage by column chromatography and/or paper electrophoresis.

EAN is the experimental disease model for one of the most common human peripheral neuropathies, infectious polyneuropathy of Guillain-Barré. The experimental disease is very similar in every regard to the human disease¹⁵ and cells sensitised to P₂ have been found in patients suffering from this disorder¹⁶. Any understanding and/or treatment of the experimental disorder may prove extremely useful in devising potential treatment for the human disease.

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S. W. BROSTOFF
S. LEVIT
J. M. POWERS

Departments of Neurology, Biochemistry and Pathology,
Medical University of South Carolina,
Charleston, South Carolina 29401

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- ¹ Waksman, B. & Adams, R. *J. exp. Med.* **102**, 213-236 (1955).
- ² Brostoff, S., Burnett, P., Lampert, P. & Eylar, E. *Nature new Biol.* **235**, 210-212 (1972).
- ³ Waksman, B. & Adams, R. *J. Neuropath. exp. Neurol.* **15**, 293-314 (1956).
- ⁴ Laatsch, R., Kies, M., Gordon, S. & Alvord, E. *J. exp. Med.* **115**, 777-788 (1962).
- ⁵ Brostoff, S. & Eylar, E. *Archs Biochem. Biophys.* **153**, 590-598 (1972).
- ⁶ Kiyota, K. & Egami, S. *J. Neurochem.* **19**, 857-864 (1972).
- ⁷ Brostoff, S. *et al. J. Neurochem.* **23**, 1037-1043 (1974).
- ⁸ Brostoff, S., Karkhanis, Y., Carlo, D., Reuter, W. & Eylar, E. *Brain Res.* **86**, 449-458 (1975).
- ⁹ Abramsky, O., Teitelbaum, D., Webb, C. & Arnon, R. *J. Neuropath. exp. Neurol.* **34**, 36-45 (1975).
- ¹⁰ Carlo, D., Karkhanis, Y., Bailey, P., Wisniewski, H. & Brostoff, S. *Brain Res.* **88**, 580-584 (1975).
- ¹¹ Brostoff, S., Sacks, H. & DiPaola, C. *J. Neurochem.* **24**, 289-294 (1975).
- ¹² Hashim, G. & Eylar, E. *Biochem. biophys. Res. Commun.* **34**, 770-776 (1969).
- ¹³ Alvord, E. & Kies, M. *J. Neuropath. exp. Neurol.* **18**, 447-457 (1959).
- ¹⁴ Wisniewski, H. M. & Bloom, B. R. *J. exp. Med.* **141**, 346-359 (1975).
- ¹⁵ Arnason, B. in *Immunological Disorders of the Nervous System* (ed. Rowland, L.) (Academic, New York, 1971), 156-177.
- ¹⁶ Sheremata, W., Colby, S., Karkhanis, Y. & Eylar, E. *Can. J. neurol. Sci.* **2**, 87-90 (1975).

Myelination of mouse axons by Schwann cells transplanted from normal and abnormal human nerves

IN mouse nerve iso- and allografts, Schwann cells transplanted from the donor animal survive, multiply within the graft and eventually, ensheath axons regenerating from the host nerve¹⁻³. This demonstration of the persistence of donor cells in nerve grafts has made possible the *in vivo* study of cell interactions in genetically-determined animal neuropathies. By combining normal and abnormal nerves, each originating from different strains of mice, a primary Schwann cell abnormality has been demonstrated in Trembler³ and Quaking⁴ mutant mice while axonal factors seem to be responsible for changes in the spinal roots of dystrophic mice^{5,6}. We report here the successful transplantation into immunologically-suppressed mice of human Schwann cells from control patients and from the sural nerve of a child with metachromatic leukodystrophy. Metachromatic leukodystrophy (MLD) is a human autosomal recessive disease due to aryl sulphatase deficiency and characterised by degeneration in the central and peripheral nervous systems with intracellular accumulations of metachromatic granules^{7,8}.

Control human sural nerves were obtained from limbs amputated because of peripheral vascular disease or trauma and from a diagnostic biopsy taken from a 10-yr-old child investigated for muscle weakness. Sections obtained from these control nerves showed no evidence of neuropathy by light microscopy. Segments of MLD nerve were obtained from a sural biopsy of a 10-yr-old girl with a 5-yr history of progressive neurological deterioration and peripheral neuropathy. The diagnosis of MLD was documented by two nerve biopsies, both of which showed the characteristic metachromatic material within Schwann cells.

Fascicles 5 mm in length were removed from the control human nerves and, in 23 normal C57 BL/6J mice, grafted between the stumps of left sciatic nerves transected at mid-thigh level. Nerve

from the patient with MLD was grafted in identical fashion into 12 other C57 BL/6J mice. All grafts were secured by 10-0 nylon sutures placed through the connective tissue surrounding the nerve. These procedures were carried out in aseptic surgical conditions within 3 h of limb amputation or biopsy, donor nerves being kept in cold Ringer's solution before grafting.

To prevent rejection of the xenografts, anti-lymphocytic serum (ALS) was prepared by injecting rabbits twice with a suspension of 10⁹ living thymocytes from C57 BL/6J mice⁹ and administered subcutaneously to the host animals in doses of 0.5 ml twice weekly. At intervals from 10 days to 3 months after grafting, host mice were killed by systemic perfusion of fixative containing 1.5% glutaraldehyde, 0.05% formaldehyde and 0.5% calcium chloride in 0.08 M Sorensen's phosphate buffer. Cross sections from the proximal and distal sciatic nerve stumps of the host and from the mid-portion of the graft were examined by phase and electron microscopy.

Soon after grafting, nerve fibres in the graft and distal stumps of the grafted nerves underwent characteristic changes of Wallerian degeneration but there was no rejection of the graft. From 3 weeks after grafting, there were increasing numbers of regenerated myelinated and unmyelinated nerve fibres within the graft and distal nerve stumps (Fig. 1a, b). Although the initial stages of regeneration and myelination were similar in grafts originating from control and MLD nerves, numerous metachromatic granules were observed within Schwann cells in the MLD grafts by 2½ months after grafting (Fig. 2a, b).

Schwann cells in the grafted segments of these regenerated nerves could have originated either by migration from the proximal nerve stump of the host or from Schwann cells indigenous to the graft. In the first instance, Schwann cells in the regenerated graft would be mouse Schwann cells; in the second, human Schwann cells would ensheath mouse axons. Although large numbers of Schwann cells do not migrate from the proximal stumps of host nerves into mouse isografts or allografts during regeneration^{1-3,10}, it is conceivable that these cells might behave differently in xenografts. Thus, to identify cells of mouse and human origin in these regenerated nerves, ALS was discontinued

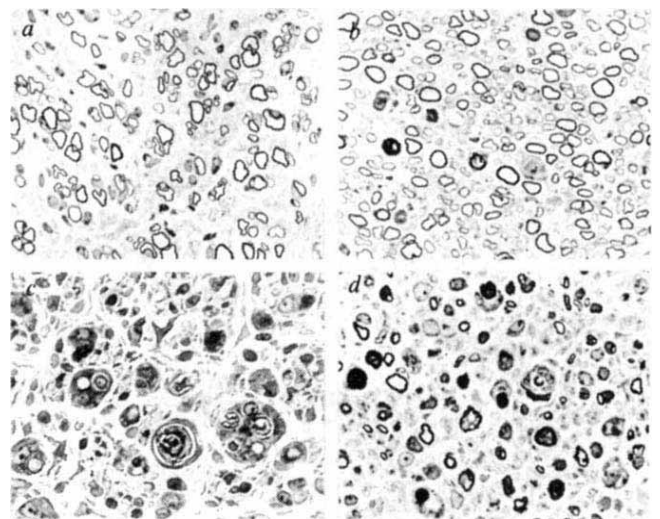


Fig. 1 Cross section of grafted segments of distal stumps of human sural nerve to mouse sciatic nerve xenografts. *a, b*, 10 weeks after grafting a segment of control human sural nerve into the sciatic nerve of an immune-suppressed mouse, axons have regenerated from the proximal stump into the graft and distal stump. At both these levels of the same regenerating nerve, there are abundant myelinated fibres. *a*, Graft; *b*, distal stump. 4 weeks after stopping ALS and 10 d after the transfer of immune cells to the host animals, a 6-week-old regenerated graft is rejected. The rejection reaction is indicated in the graft (*c*) by myelin debris, increased cellularity and a complete loss of myelinated fibres. In the distal stump of the same nerve (*d*) many myelinated fibres are preserved although some show signs of Wallerian degeneration, presumably due to axonal damage at the level of the graft. (Phase contrast photomicrographs ($\times 660$)).

in a group of mice from 4 weeks to 2 months after grafting. From 2 to 3 weeks after discontinuation of ALS these mice also received intravenously 10^8 lymphoid cells prepared from the spleen and lymph nodes of syngeneic mice hyper-immunised against tissue antigens by intravenous injection of 10^7 – 10^8 human leukocytes twice weekly for 3 weeks. This cell transfer was used to abrogate the xenograft acceptance in the recipient animal and to ensure a more rapid rejection of all 'foreign' cells¹¹. Although discontinuation of ALS will cause xenograft rejection, the additional immune cell transfer permits a more accurate timing of rejection for histological studies.

Ten mice in which ALS had been discontinued were examined for 4–81 d after immune cell transfer. On d 8, early rejection was indicated by small accumulations of mononuclear cells in the nerve grafts. By d 10, there was an intense reaction in the grafted segments (Fig. 1c). Schwann cells of both myelinated and unmyelinated fibres were surrounded, penetrated and eventually replaced by mononuclear cells (Fig. 3a). Perineurial fibroblasts were also rejected in the grafts. During this acute phase of rejection, some axons became totally denuded of Schwann cells and were surrounded only by basal lamina (Fig. 3b).

During graft rejection, many fibres in the distal stumps remained myelinated, showing that the continuity of their axons was preserved. But, a certain number of axons must have been interrupted, presumably in the graft, because fibres undergoing Wallerian degeneration were observed in distal stumps of the same nerves (Fig. 1d).

In two animals allowed to survive the period of rejection for more than 2 months, the segments of nerves at the site of the original graft again contained large numbers of myelinated fibres. Presumably, migration of mouse Schwann cells from the stumps of the host nerves was responsible for the reappearance of these myelinated fibres. This migration may have been facilitated and guided by the axons and empty basal lamina which survived rejection.

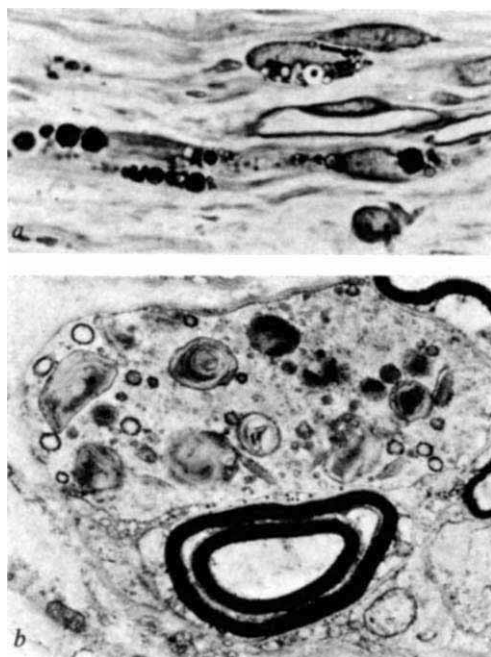


Fig. 2 Segments of human MLD sural nerve grafted into mouse sciatic nerve. *a*, After 2½ months, axons from the mouse nerve stump have regenerated into the graft and are associated with Schwann cells which contain myelin ovoids and numerous small granules. These granules, which stain metachromatically with cresylviolet, were confined to the graft and were not observed in the proximal and distal nerve stumps. (Longitudinal section, phase micrograph ($\times 1,200$)). *b*, Lamellated and amorphous intracellular inclusions are present in the mid-portion of a human MLD to mouse sciatic nerve graft. (Electron micrograph ($\times 13,500$)).

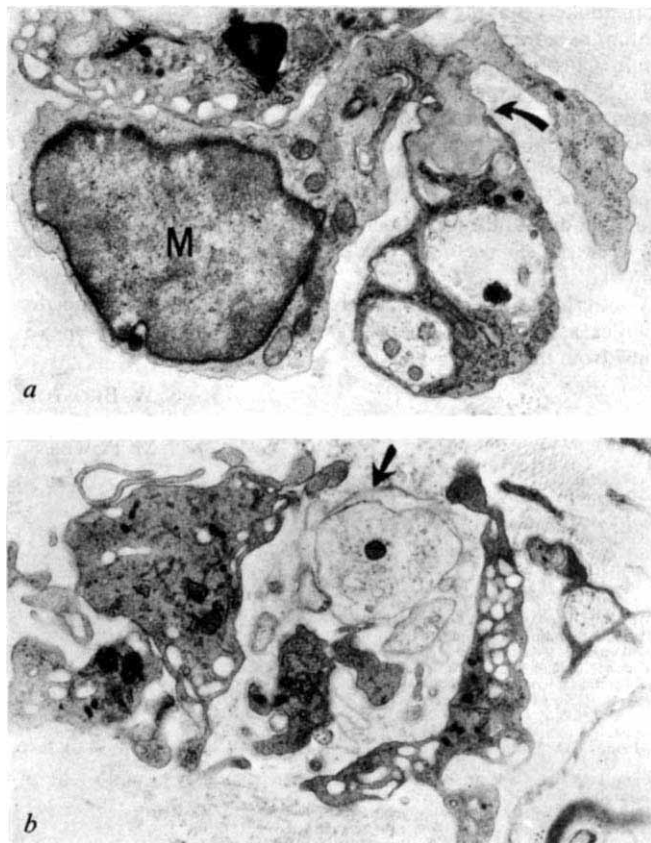


Fig. 3 Details of rejection of the nerve graft shown in Fig. 1c. *A*, cytoplasmic process from a mononuclear cell (M) has partially penetrated the basal lamina of an unmyelinated fibre (arrow). *b*, One large and several small axons persist within the basal lamina (arrow) of a rejected Schwann cell. Macrophage processes are present on both sides of the residual basal lamina. (Electron micrograph ($\times 9,800$)).

The selective rejection of most of the sheath cells in the regenerated grafts establishes that the grafted segments of these nerves still contained fibroblasts and Schwann cells foreign to the mouse. Thus, regenerated nerve fibres within the grafts must represent a combination of mouse axons and human Schwann cells. The presence of metachromatic granules in grafts originating from the sural nerve of a patient with MLD 2½ months after grafting into mouse nerves is additional evidence of the presence of human Schwann cells in the regenerated xenografts. This finding also suggests that the grafted MLD sheath cells continue to be deficient in aryl sulphatase during nerve regeneration in the mouse and may be unable to utilise this enzyme from the host.

These studies in nerve xenografts have shown that (1) human Schwann cells can ensheath and myelinate regenerating mouse axons; (2) the perineurium of the regenerated grafts contain human fibroblasts; (3) during rejection of regenerated grafts, the immune response is directed against fibroblasts as well as Schwann cells of myelinated and unmyelinated fibres; axons may be affected secondarily¹²; (4) if, after regeneration across a graft, the xenogenic sheath cells are rejected, they can be replaced by the host's own Schwann cells which migrate along the re-established pathways formed by axons and basal lamina tubes; (5) human neuropathies may be reproduced by transplanting fascicles from the patient's nerve into immune-suppressed animals.

Thus, the present *in vivo* combinations of human and animal cells may help in the investigation of biological mechanisms and permit a better definition of the role of axons and Schwann cells in normal and pathologic human nerves. Human Schwann cells transplanted into laboratory animals are now amenable to experimental manipulations which may include enzyme replacement and immunological studies.

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A. J. AGUAYO*
J. KASARJIAN
E. SKAMENE
P. KONGSHAVN
G. M. BRAY

*Divisions of Neurology and Immunology,
The Montreal General Hospital and McGill University,
Canada*

Received 8 June; accepted 5 July 1977.

*To whom correspondence should be addressed at: Division of Neurology, Montreal General Hospital, 1650 Cedar Avenue, Montreal, Quebec H3G 1A4 Canada

- ¹ Aguayo, A. J., Epps, J., Charron, L. & Bray, G. M. *Brain Res.* **1**, 1-20 (1976).
- ² Aguayo, A. J., Charron, L. & Bray, G. M. *J. Neurocytol.* **5**, 565-573 (1976).
- ³ Aguayo, A. J., Attiwell, M., Trecarten, J., Perkins, S. & Bray, G. M. *Nature* **265**, 73-75 (1977).
- ⁴ Aguayo, A. J., Mizuno, K. & Bray, G. M. *J. Neuropath. exp. Neurol.* **36**, 595 (1977).
- ⁵ Bray, G. M., Cullen, M. J., Aguayo, A. J. & Rasminsky, M. *Neurology* **27**, 362 (1977).
- ⁶ Bray, G. M., Aguayo, A. J. & Perkins, S. *Can. J. Neurol. Sci.* (in the press).
- ⁷ Austin, J. M., Armstrong, D. & Shearer, L. *Archs. Neurol.* **13**, 593, (1965).
- ⁸ Suzuki, K., Suzuki, K. & Chen, G. C. *J. Neuropath. exp. Neurol.* **26**, 537-550 (1967).
- ⁹ Levey, R. H. & Medawar, P. B. *Proc. natn. Acad. Sci. U.S.A.* **56**, 1130-1137 (1966).
- ¹⁰ Charron, L., Aguayo, A. J. & Bray, G. M. *Can. J. Neurol. Sci.* **3**, 143 (1976).
- ¹¹ Steinmuller, D. *Transpl. Proc.* **2**, 438 (1970).
- ¹² Madrid, R. C. & Wisniewski, H. M. *J. Neurocytol.* **6**, 103-117 (1977).

Two mechanisms for positive inotropism of low-K Ringer solution in bullfrog atrium

It has long been known that a reduction of K concentration in external fluid immediately potentiates the twitch contraction of frog heart¹. Microelectrode studies^{2,3} have revealed a hyperpolarisation of the membrane and a prolongation of the action potential, which was thought to be a cause of the potentiation of contraction. But, when the muscle is soaked in K-depleted fluid the membrane Na/K ATPase and the activity of the Na pump are inhibited⁴ and Na ions progressively accumulate within the cell⁵. In myocardium, the accumulation or rise of [Na]_i produces a secondary increase of [Ca]_i due to Na-Ca exchange diffusion and the consequent positive inotropic effect^{6,7}. Thus, Ehara⁸ has demonstrated the presence of a delayed potentiating effect of low-K Ringer solution possibly due to Na-pump inhibition in the frog ventricle as well as its immediate inotropic effect. We have tried to clarify further the nature of these inotropic actions. We show here that, in the frog atrium, augmentation of Ca-inward current (I_{Ca}) and increase of I_{Ca} -independent tonic tension, related to Na-Ca exchange, are responsible for the immediate and delayed inotropic effects under voltage clamp, respectively.

Thin muscle bundles (diameter 0.4-0.5 mm) isolated from the right atrium of the bullfrog, *Rana catesbiana*, were used. The membrane potential, current and tension were measured in voltage-clamped and unclamped conditions with the conventional double-gap method⁹, in which the width of the central test chamber was about half (0.3-0.4 mm) of the space constant¹⁰. With this apparatus gap action potentials (AP) of more than 100 mV were commonly obtained and the short circuiting factor was greater than 0.8 for both gaps. The central chamber was initially perfused with normal Ringer containing 2.5-4.0 mM K until the twitch tension became steady, and then the solution was replaced with low-K Ringer (0-0.16 mM K). All experiments were performed at a constant temperature, 16-17 °C.

In unclamped conditions, K-depleted Ringer solution produced biphasic positive inotropic effects in some atrial preparations (Fig. 1a) but not as often as in the ventricle⁸. The partially fused effects seemed much faster (five to six

times) in the former than in the latter, probably due to thinness of the muscle fibres and scattering of the bundles in the atrium. The immediate and late potentiations, however, could be discriminated in the presence of ouabain (10^{-6} M, Fig. 1c) which eliminated the late potentiating effect. The initial inotropic effect was accompanied by a hyperpolarisation of the membrane, a rise in threshold, and an increase in amplitude and duration of AP. During the late inotropic effect, on the other hand, a gradual depolarisation plus lowering and prolongation of AP were produced together with a gradual increase of membrane slope resistance (Fig. 1d). Automaticity was often elicited during this later phase (Fig. 1b), and augmented twitch contraction showed a special pattern of fast tension development and slow relaxation. In the final depressive stage of contraction, the basal tension increased and the AP was further suppressed; and after a few hours of soaking in K-depleted Ringer, addition of K ions (4 mM) produced recovery, and hyperpolarisation instead of depolarisation of the membrane. This suggests activation of the electrogenic Na pump (Fig. 1e).

Voltage-clamp studies during the initial 2-5 min after K-depletion disclosed that considerable potentiation of contraction still occurred in response to constant depolarising pulses. The fast Na-inward current (I_{Na}), though not accurately measurable, was virtually unchanged during this period (see Fig. 2c), however the slow inward current in the presence of tetrodotoxin (TTX) (5×10^{-7} g ml⁻¹) and the Ca-inward current (I_{Ca}) isolated in Na-free sucrose Ringer solution were markedly prolonged after K-depletion. The

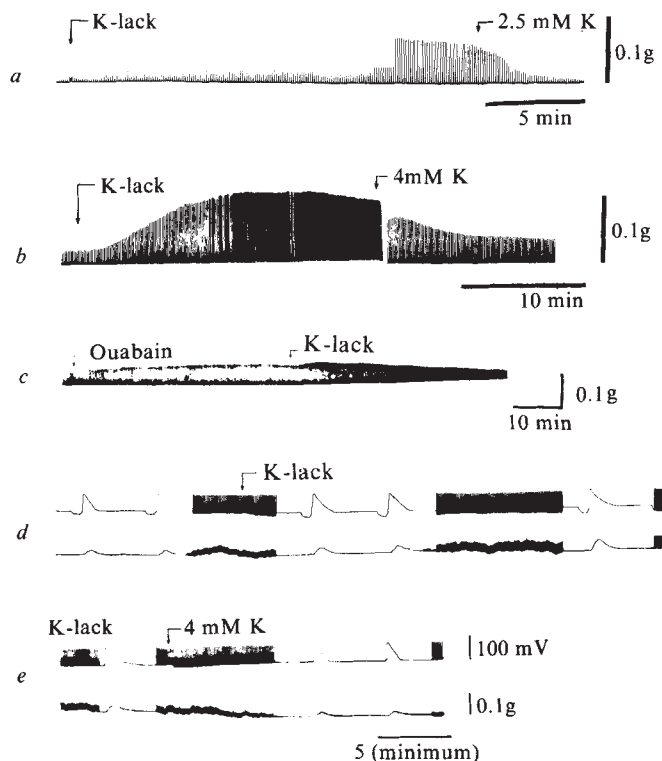


Fig. 1 Immediate and delayed positive inotropic effects of K-free Ringer on bullfrog atrium. *a*, Biphasic inotropic responses. *b*, Monophasic inotropic response with arrhythmia at later phase. *c*, Effects of K-depletion in the presence of ouabain (10^{-6} M). *d*, Effects of K-depletion on membrane potential and contractility. A hyperpolarising pulse was given before each action potential. *e*, Effects of administration of 4 mM K at the depressive stage 5 h after K-depletion. Note increase of membrane slope resistance and hyperpolarisation followed by gradual depolarisation in (*c*) and appearance of hyperpolarisation after administration of K in (*e*).

prolongation was mainly due to a long delay of the inactivation process (Fig. 2*a,b*). The leaky inward and outward currents decreased as expected (Fig. 2*c*). But, the voltage-current relationships obtained in Na-free Ringer solution, in which the anomalous rectification was completely eliminated, confirmed the initial enhancement of I_{Ca} after K-depletion (Fig. 2*d*). The experiments also clarified a concomitant augmentation of the I_{Ca} -dependent tension (not shown). These observations clearly indicate that the initial inotropic effect of K-depletion is not simply dependent on prolongation of the AP due to decrease of leaky currents but also on an augmentation of I_{Ca} and I_{Ca} -dependent tension.

Meanwhile, during the late inotropic phase more than 10 min after K-depletion, the fast and slow inward currents

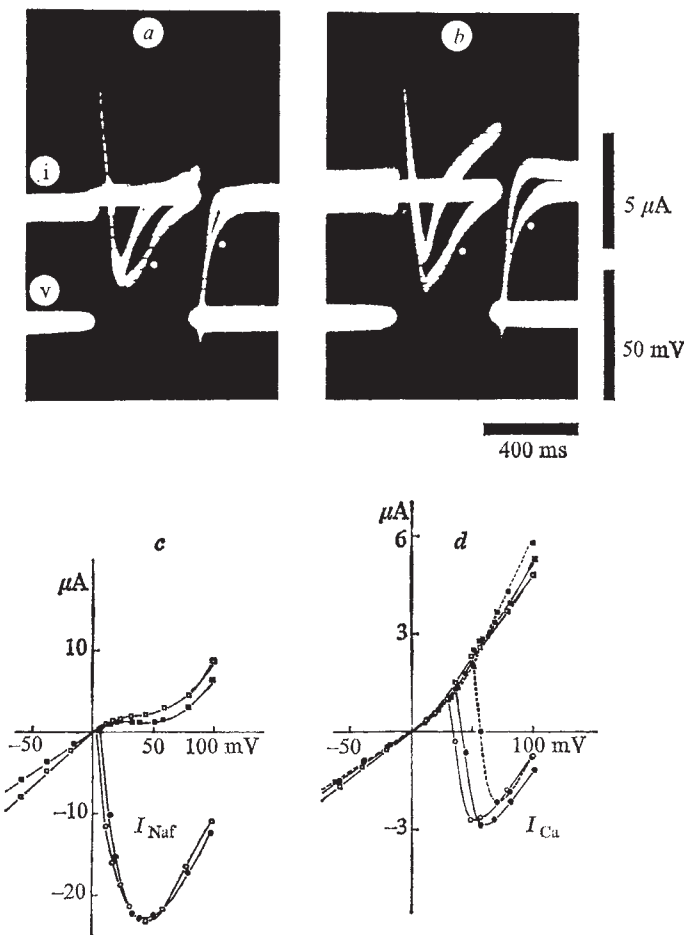


Fig. 2 Initial effects of K-depletion on membrane currents in voltage clamp. The membrane potential was held at the gap resting potential and rectangular pulses were applied at intervals exceeding 10 s. *a*, Superimposed records of Ca-inward currents before, and 5 min after K-depletion ($\circ$) in response to the same depolarising pulses (47 mV, 400 ms) given from the holding potential level (-70 mV). Data were obtained in Na-free sucrose Ringer solution throughout. *b*, Similar superimposed records taken 6 min after K-depletion ($\circ$) and 2 min after washing with normal Ringer solution (4 mM K). Note prolongation of Ca inward current after K-depletion in (*a*), and depression of current after washing in (*b*). Similar changes are observable in the tail currents. *c*, Voltage-current relationships before and after K-depletion. Long depolarising pulses (1.0 s) were used. $\circ$, $\square$, Control. $\bullet$, $\blacksquare$, 10 min after K-lack. As (*c*), but in Na-free sucrose Ringer solution. $\circ$, $\square$, Control. $\bullet$, $\blacksquare$, 3 min after K-lack. $\bullet$, $\blacksquare$, 10 min after K-lack. In (*c*), both inward and outward terminal currents (squares) were markedly inhibited while fast dynamic inward currents ($\circ$) were unaltered. In (*d*), no anomalous rectification was observable due to Na-free condition. Note apparent enhancement of I_{Ca} ($\circ$) at 3 min after K-depletion.

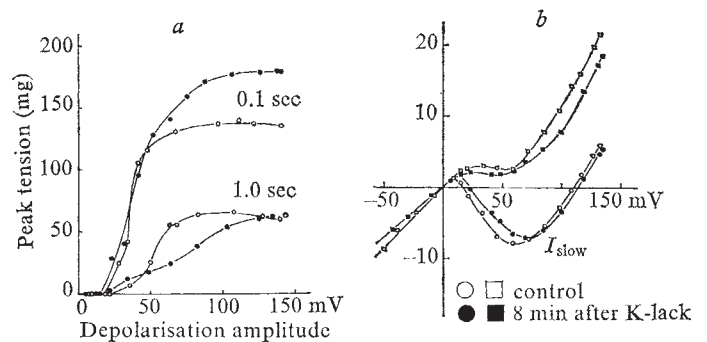


Fig. 3 Late effects of K-depletion on membrane currents and tension components under voltage clamp. *a*, Voltage-tension relationships. Short and long depolarising pulses (0.1 s and 1.0 s) were used and peak tension before ($\circ$), and 10–15 min after ($\bullet$) K-depletion were plotted against amplitude of pulses. The contractile tensions in response to short pulse (mainly I_{Ca} -dependent tension) were inhibited while those for long and strong pulses (largely I_{Ca} -independent tension) were augmented. *b*, Voltage-current relationships before ($\circ$, $\square$) and 8–10 min after ($\bullet$, $\blacksquare$) K-depletion in presence of tetrodotoxin (5×10^{-7} g ml $^{-1}$). The squares mark terminal currents and the circles, peak inward currents. Long pulses (1.0 s) were used. The slow inward current and the inward as well as outward terminal currents were depressed.

were gradually depressed (not shown). The I_{Ca} -dependent phasic component of tension diminished, while the I_{Ca} -independent tonic component was markedly augmented (Fig. 3*a*). Voltage-current relationships during these later phases also showed a decrease of the slow inward current (Fig. 3*b*) as well as the isolated I_{Ca} (Fig. 2*d*). Depression of the leaky inward and outward terminal currents persisted. Those characteristics may well account for the typical configuration of the action potential: depressed overshoot and plateau followed by a long tail (Fig. 1*e*). The marked reduction of electrogenic outward current by inhibition of Na pump observed in sheep Purkinje fibres¹¹ was not seen in the frog atrium, although a presence of an electrogenic potential was noticed in the final depressive stage of contraction. Thus, the principal cause of the late positive inotropic effect of low-K solution was augmentation of I_{Ca} -independent tonic tension and prolongation of the action potential.

It should be noted that these initial and late effects of K-depletion seemed to be quite similar to those of ouabain^{12,13}. Although the mechanism of therapeutic action of ouabain is not yet known, one possibility suggested by Baker *et al.*¹⁴ and Langer¹⁵ is that inhibition of the Na pump and the consequent increase of [Na]_i may allow an accumulation of Ca into the cell through the Na-Ca exchange mechanism. In fact, ouabain was shown by Vassort¹⁶ to increase only the I_{Ca} -independent tonic tension in frog atrium, which largely depends on Na-Ca exchange. This may be compatible with the late inotropic effect of K-depletion in our case where the Na pump was also inhibited⁸. Another possibility has been proposed^{17,18}, however—a therapeutic low dose of ouabain may produce net stimulation rather than inhibition of the Na/K-ATPase. Blood¹⁹ used doses of ouabain (5×10^{-10} to 10^{-7} M) that exert a definite positive inotropic action on Purkinje fibre. These doses were found by Cohen *et al.*²⁰ to increase the K gradient across the membrane, probably as a consequence of stimulation of the Na pump. These effects may well correspond to the initial effects of K-depletion, at least in the condition in which [K]_o decreases. The inotropic action of cardiac glycosides is probably not mediated by an increase of I_{Ca} , but rather, is accompanied by its decrease^{12,13}; however, the effects of a low dose on I_{Ca} have not been examined. In any case, the appearance of I_{Ca} augmentation after K-depletion

may explain some part of the long known antagonistic action of Ca and K on myocardial contractility.

MASAYOSI GOTO

YASUO TSUDA

ATSUKO YATANI

Department of Physiology II,
Faculty of Medicine, Kyushu University,
812 Fukuoka, Japan

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- ¹ Ringer, S. J. *J. Physiol., Lond.* 4, 29–42 (1883).
- ² Brooks, C. M., Hoffman, B. F., Suckling, E. E. & Orias, O. *Excitability of the Heart* (Grune & Stratton, New York, 1955).
- ³ Brady, A. J. & Woodbury, J. W. *Ann. N. Y. Acad. Sci.* 65, 687–692 (1957).
- ⁴ Bonting, S. L. in *Membrane and Ion Transport 1* (ed. Bittar, E. E.) 257–363 (Wiley London, 1970).
- ⁵ Keenan, M. J. & Niedergierke, R. J. *J. Physiol., Lond.* 188, 235–260 (1967).
- ⁶ Reuter, H. & Seitz, N. J. *J. Physiol., Lond.* 195, 451–470 (1968).
- ⁷ Poole-Wilson, P. A. & Langer, G. A. *Circulat. Res.* 37, 390–395 (1975).
- ⁸ Ehara, T. *Jap. J. Physiol.* 24, 329–342 (1974).
- ⁹ Goto, M., Wada, Y. & Saito, M. *Jap. J. Physiol.* 24, 359–375 (1974).
- ¹⁰ Brown, H. F., Noble, D. & Noble, S. J. *J. Physiol., Lond.* 258, 615–629 (1975).
- ¹¹ Isenberg, G. & Trautwein, W. *Pflügers Arch. ges. Physiol.* 350, 41–54 (1974).
- ¹² Greenspan, A. M. & Morad, M. *J. Physiol., Lond.* 253, 357–384 (1975).
- ¹³ McDonald, T. F., Nawrath, H. & Trautwein, W. *Circulat. Res.* 37, 674–682 (1975).
- ¹⁴ Baker, P. F., Blaustein, M. P., Hodgkin, A. L. & Steinhardt, R. A. *J. Physiol., Lond.* 200, 431–458 (1969).
- ¹⁵ Langer, G. A. *J. gen. Physiol.* 50, 1221–1239 (1967).
- ¹⁶ Vassort, G. *Pflügers Arch. ges. Physiol.* 339, 225–240 (1973).
- ¹⁷ Okita, G. T., Richardson, F. & Roth-Schechter, B. F. *J. Pharmac. exp. Ther.* 185, 1–11 (1973).
- ¹⁸ Peters, T., Raben, R. M. & Wasserman, O. *Eur. J. Pharmac.* 26, 166–174 (1974).
- ¹⁹ Blood, B. E. *J. Physiol., Lond.* 251, 69–70P (1975).
- ²⁰ Cohen, I., Daut, J. & Noble, D. *J. Physiol., Lond.* 260, 75–103 (1976).

Sarcomere shortening in striated muscle occurs in stepwise fashion

DURING the development of a high resolution light diffractometer to study sarcomere dynamics, we have observed an unexpected feature of the pattern of sarcomere shortening. We report here that sarcomere shortening during contraction seems to occur in a series of successive bursts punctuated by short but well defined periods during which motion has virtually ceased. Furthermore, it seems that this stepwise pattern of shortening may occur synchronously over many contiguous sarcomeres.

Cardiac and skeletal muscle specimens were mounted in an apparatus (Fig. 1), components of which have been described¹. The muscle was transilluminated with laser light (Spectra Physics He-Ne laser, Model 120, $\lambda=0.6238 \mu\text{m}$). The diffraction pattern was collected at the rear focal plane of a water immersion objective (Zeiss $\times 40$, effective numerical aperture 0.5). Sarcomere length was computed from the spacing between diffracted orders using the Fourier relationship.

We use three methods of analysis (Fig. 1). With each method the diffraction pattern was first compressed along the length of each order with a cylindrical lens; this increased intensity and averaged optical noise. The compressed pattern was then projected on the three sensors. The principal sensor was a 128-element photodiode array (Reticon RL-128A; diode spacing $50 \mu\text{m}$; aperture $25 \mu\text{m} \times 430 \mu\text{m}$). The array was scanned 5 times per ms. Each scan generates a profile of intensity against spatial frequency of the striation pattern. Sarcomere length was computed during each scan from the distance between the zeroth order peak and the computed median of the first order (T.I. and G.H.P. in preparation). The sarcomere length signal was calibrated with a series of gratings; the accuracy was within 1% between $1.6 \mu\text{m}$ and $3.3 \mu\text{m}$. Resolution was approximately 5 nm.

In the second method a slice of the diffraction pattern was projected through a slit (width 0.9 mm) on to film in a kymographic camera². The time resolution was limited to

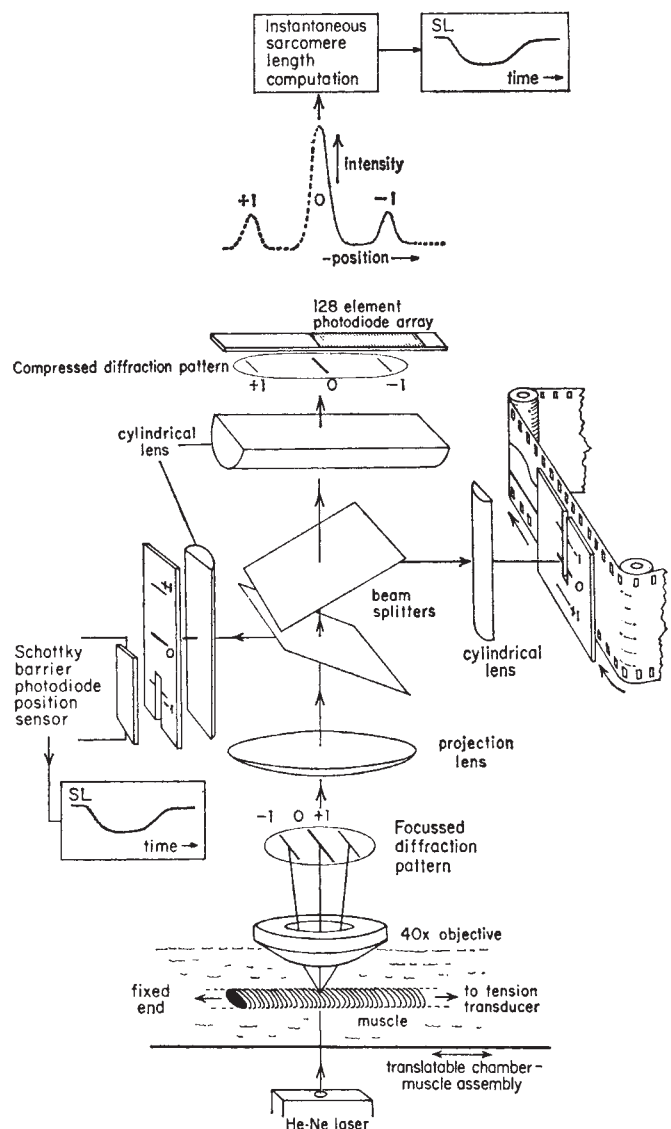


Fig. 1 Apparatus used to measure sarcomere length with high time and length resolution. The diffraction pattern of the muscle collected at the rear focal plane of the objective lens is projected onto three sensors for analysis. Sarcomere length is derived from the spacing between bands of the diffraction pattern.

about 4 ms. Densitometry measurements were made at intervals of 2 ms along the film, and a plot of intensity against spatial frequency was obtained at each location. The position of the median of the first order was determined by planimetric integration. Sarcomere length was computed from the spacing between the median and the zeroth order peak. From these computations a plot of sarcomere length against time was obtained.

In the third method, one of the first orders was projected through a slit onto a Schottky barrier photodiode (United Detector Tech. SC/25). The photodiode has a cathode at each end, and the difference of cathode currents is directly related to the position of the centroid of the light spot. Sarcomere length was computed from this current difference, giving a continuous output with a bandwidth of about 100 kHz. Calibration error was within 3% throughout the relevant range of striation spacings.

Representative examples of the time course of sarcomere shortening during contraction obtained with the photodiode array are shown in Fig. 2. These are records of 'internal' shortening during isometric twitches (though comparable phenomena were observed in non-isometric twitches and

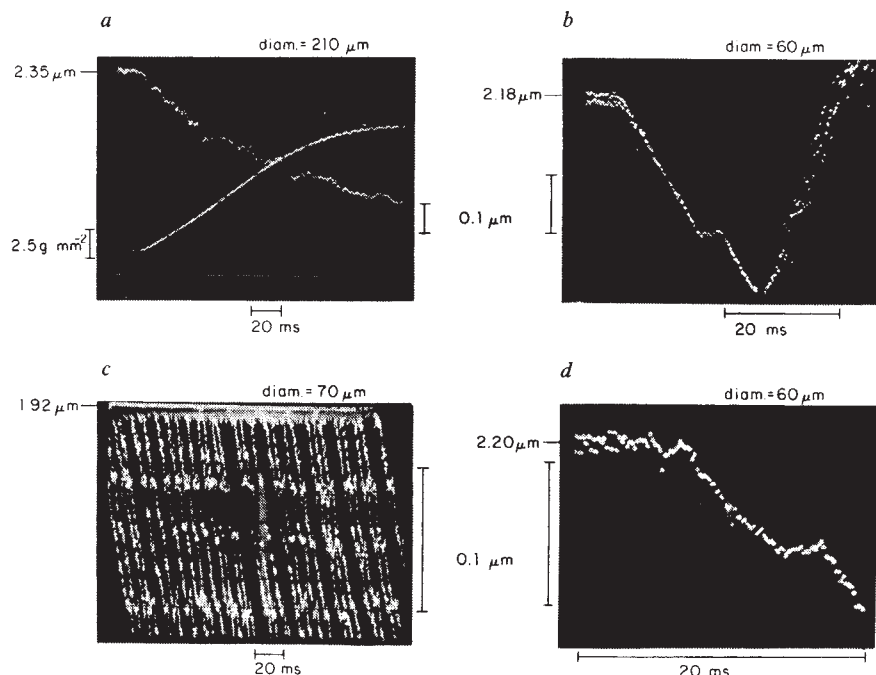


Fig. 2 Records of the time course of sarcomere length during contraction, taken from rat papillary (*a*), frog skeletal (*b* and *d*) and rat trabecular (*c*) muscle. All records were obtained using the photodiode array. In (*a*) the tension record and the stimulus pulse are shown as well. Note the stepwise shortening pattern observed early in the contraction. In the twitch depicted in (*b*) only one step is seen, lasting about 4 ms. The trace becomes noisy during relaxation as a consequence of the breakup of the diffraction pattern, as reported previously². The Records in (*c*) are from a series of contractions (each displaced successively rightward) and demonstrate the repeatability of the stepwise pattern. Because of the expansion of the sarcomere length scale relative to the time scale, each plateau appears as a bright spot on the trace. Record (*d*) was obtained with high time and sarcomere length sensitivity on the oscilloscope, so that each sarcomere length determination showed up distinctly. Only the early portion of contraction is shown. The transition between shortening and plateau (right side of panel) occurs within several hundred microseconds, the time for one or two sarcomere length determinations.

tetani). In Fig. 2*a* shortening commenced after a latent period following stimulation and continued for several milliseconds. Then, abruptly, shortening ceased and did not resume for 8–10 ms (a period encompassing 40–50 determinations of sarcomere length). This stepwise shortening pattern was repeated several times during the early phase of contraction. Later in the cycle the signal became irregular and failed to show distinct plateaus. Thinner specimens often showed plateaus throughout the shortening phase of contraction, possibly because of their relatively sharper diffraction patterns.

Figure 2*b* shows a shortening pattern obtained from a single fibre of skeletal muscle. Only one distinct plateau is evident. Although other records did show multiple plateaus, we usually found fewer and shorter plateaus than in comparable contractions of cardiac muscle. The series of shortening traces in Fig. 2*c* was obtained from successive contractions in the same optical region of a trabecular specimen. As this record shows, plateaus occurred at approximately the same sarcomere length in every contraction. In different regions along the specimen, however, different stepwise shortening patterns were usually found, and in some regions no steps were evident.

Figure 2*d* shows a portion of a shortening trace recorded with high sensitivity to illustrate the abruptness of the transition between shortening and plateau. Transitions were usually complete within 1 ms.

Stepwise shortening patterns might arise artefactually from at least two potential sources. The specimen could translate across the optical field from a shortening to a static region. We have seen such patterns, however, in regions which translated only several per cent of the field diameter throughout contraction. Another potential source of artefact is the photodiode array and associated electronics. To test this, we recorded some diffraction patterns simultaneously using both the photodiode array and the kymographic technique outlined in Fig. 1.

Analysis of the kymographic records was not entirely satisfactory, as the limited time resolution precluded the delineation of any sharp corners in the shortening pattern. Furthermore, faithful representation of the shortening pattern placed high demands on the accuracy of the planimetric determinations; relatively minor errors produce enough scatter to blur fine details of the shortening pattern.

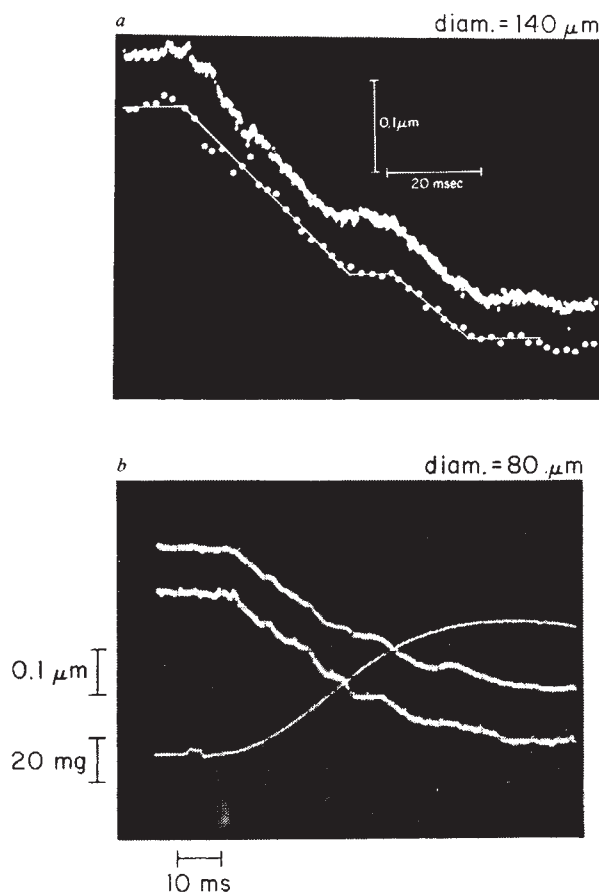


Fig. 3 Time course sarcomere length (from rat trabecular muscle) measured simultaneously with the photodiode array (continuous curve) and with the kymographic technique (points). The records are vertically displaced for clarity. Initial sarcomere length was 2.3 μ m. A plateau seems to be present in both records at a point just beneath the sarcomere length scale; however, the restricted time and space resolution of the kymographic technique limits the confidence with which the existence of a plateau can be inferred. *b*, Time course of sarcomere length changes measured simultaneously with the photodiode array (upper trace) and the Schottky barrier photodiode (middle trace). Records are vertically displaced for clarity. Initial sarcomere length was 2.1 μ m. The tension record, with superimposed stimulus artefact, is shown beneath.

Nonetheless, these records showed a fair degree of correspondence with those obtained with the photodiode array (Fig. 3a).

Because we judged the correspondence between the two results to be inconclusive, we adopted still another methodology, the Schottky barrier photodiode (Fig. 1). As Fig. 3b shows, the records obtained with this analogue technique show a stepwise shortening pattern similar to that obtained simultaneously with the photodiode array. The similarity of the records convinces us that the spacing between the zeroth and first order changes in stepwise fashion.

If so, the shortening behaviour among a large fraction of the sarcomeres in the optical field must have been co-ordinated; otherwise the sharp corners in the shortening trace could not have been observed. Since each field covered approximately 180 μm , the region of spatial coherence must have extended over at least the major fraction of this dimension. On the other hand, we found regional variations along the muscle of the step-like shortening patterns, so that the region of spatial coherence apparently did not extend far beyond the dimension of an optical field.

Plateaus in the shortening patterns could arise in at least three ways: (1) most sarcomeres in an optical field could undergo no length change during the period of the plateau; (2) populations of lengthening and shortening sarcomeres could cancel one another, or (3) all sarcomeres might shorten continually, but the contribution to the diffraction pattern of the shorter sarcomeres could diminish gradually (due perhaps to increasing misregistration of sarcomeres), as the contribution of the longer sarcomeres gradually increases.

Possibility (3) requires that at least two distinct sarcomere populations exist within the optical field, that the degree of spatial coherence of each be cyclic and out of phase with the other, and that the cycles occur at specific sarcomere lengths. The combination of these *ad hoc* assumptions seems too unrealistic to make this explanation likely. Possibility (2) demands that a fraction of sarcomeres in the field suddenly begins lengthening just rapidly enough to cancel the contribution of shortening sarcomeres. By the end of a plateau the two populations ought to have diverged by an amount detectable on the kymographic records; we observed no such divergence. Thus the first possibility—that most sarcomeres in the field suddenly cease shortening—remains the most likely explanation of the plateaus in the stepwise shortening pattern.

If our observations withstand the test of time, any proposed molecular theory of contraction would have to incorporate a mechanism to account both for stepwise shortening in a single contractile unit, and for synchronisation of this stepwise pattern among a large number of contiguous contractile units. It is not immediately obvious how the cross-bridge theory as now envisaged^{3,4} might satisfy such a requirement.

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GERALD H. POLLACK
TATSUO IWAZUMI
HENK E. D. J. TER KEURS*
ERWIN F. SHIBATA

Departments of Anesthesiology and
Bioengineering, RN-10
University of Washington,
Seattle, Washington 98195

Received 7 April; accepted 5 July 1977.

* Permanent address: Department of Cardiology, University of Leiden, The Netherlands.

¹ Pollack, G. H. & Krueger, J. W. *Eur. J. Cardiol.* 4, Suppl. 53-65 (1976).

² Cleworth, D. R. & Edman, K. A. P. *J. Physiol.* 227, 1-17 (1972).

³ Huxley, H. E. *Science* 164, 1356-1366 (1969).

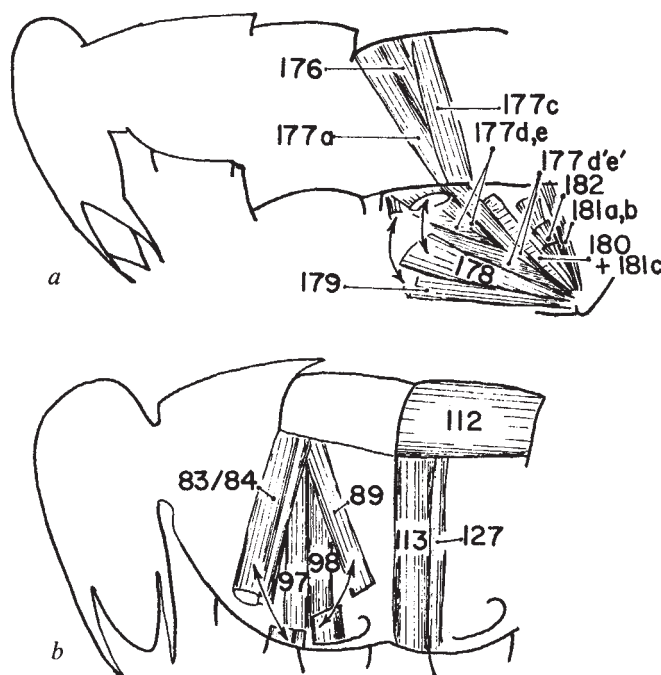
⁴ Huxley, A. F. *J. Physiol.* 243, 1-43 (1974).

Does electrophoresis of cockroach muscle proteins detect recognition molecules?

IN some animals, particularly amphibia and insects, normal function is ultimately restored in a limb which has been denervated. In the cockroach it has been shown that identified motoneurons reinnervate the same leg muscles as they did before the nerve was sectioned^{1,2}. A possible explanation is that each muscle has specific molecules by which ingrowing nerves are able to recognise their appropriate destination. Recently, Denburg³, using sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis has shown that these cockroach muscles have distinct gel protein patterns which he suggests represent specific recognition molecules. Alternatively, the differences may arise because the motor neurones he considers are physiologically different types, 'fast' and 'slow'. To test this interpretation we have examined a number of other cockroach muscles using Denburg's methods. All the muscles have patterns which may be grouped into the same categories as Denburg's even though they are innervated by different neurones. Where the innervation by 'fast' and 'slow' neurones is known the correspondence with our data is good. Furthermore, locust flight muscles with solely 'fast' innervation all have identical patterns. We conclude that gel electrophoresis patterns demonstrate a characteristic of the type of innervation rather than representing specific recognition molecules.

On theoretical grounds it seems unlikely that a procedure as crude as SDS gel electrophoretic analysis of whole muscle extract should reveal molecules concerned in specific recognition by motoneurons. One might reasonably expect that every

Fig. 1 *a*, Positions of the muscles in the cockroach which have been examined by SDS-polyacrylamide electrophoresis (see Fig. 2). The muscles are numbered according to Carbonell⁵. Muscles 161, 163 and 167 lie close to 177a and have been omitted for clarity. Muscle 174 lies close to 176 and is omitted also. *b*, Positions of the flight muscles in the locust which have been examined (see Fig. 3).



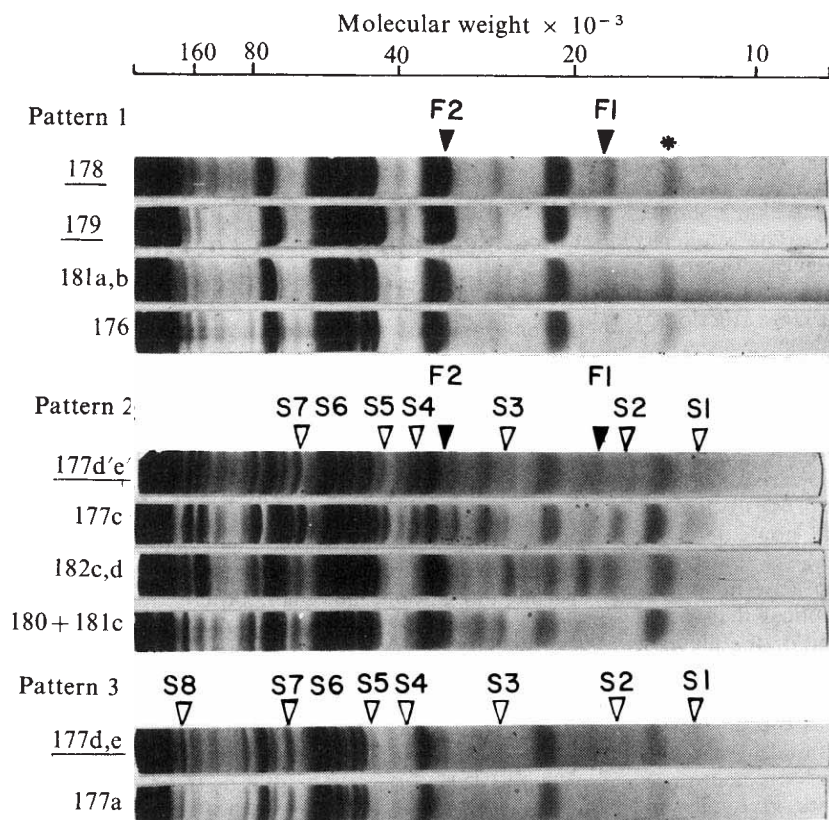


Fig. 2 SDS-polyacrylamide gels of the cockroach muscles shown in Fig. 1a, stained with Coomassie Blue. The numbers of the muscles examined by Denburg are underlined and those bands corresponding to the R_f values of his bands F1-2 and S1-8 are indicated. An additional band (labelled *) not described by Denburg is also indicated. This figure demonstrates that the patterns 1-3 from the muscles Denburg examined are not unique to muscles innervated by motoneurons 28 and 52, which argues strongly against their representing specific recognition molecules. Isolated muscles were homogenised in an appropriate volume of 0.01 M Tris-HCl (pH 7.4), 20% glycerol, 1% SDS and 5% 2-mercaptoethanol and heated in a boiling water bath for 5 min. Aliquots of these solutions were applied to 10% polyacrylamide gels which were run in the presence of 0.2% SDS, stained with Coomassie Blue, and destained using the methods of Fairbanks *et al.*⁴. The gels have been severed at the dye marker band.

structural and soluble protein of the cell would be represented in the extract and yet the largest number of bands we have observed using Denburg's technique is 33. Clearly only those proteins with the largest representation in the cell are being made visible by the method and it would seem improbable that specific recognition molecules, which have previously evaded identification would be in the top 33.

Denburg found that the metathoracic coxal depressor muscles 178 and 179, which are innervated by cell 28, have a banding pattern different from that of muscles 177d and 177e which are innervated by cell 52. The gel patterns for muscles 177d' and 177e', which are innervated by both motoneurons, have features common to both types (Figs 1a and 2). He considers that particular bands in these patterns represent distinctive macromolecules by which ingrowing nerves are able to recognise their appropriate muscle. Our alternative explanation is that the gel protein pattern for muscles innervated by cell 28 is different from those of muscles innervated by cell 52 simply

because cell 28 is a 'fast' motoneurone, while cell 52 is a 'slow' motoneurone. If this interpretation is correct then all muscles with 'fast' innervation should show the one pattern, those with 'slow' the other, and muscles innervated by both kinds of neurone should show the sum of the two patterns. Unfortunately Denburg only analysed the muscles which are innervated by cells 28 and 52 and he did not test this possibility.

We have examined 14 cockroach muscles using the same method of SDS-polyacrylamide gel electrophoresis^{3,4} and have obtained banding patterns very similar to his for the muscles he analysed (Fig. 2). Muscles 178 and 179 have a pattern which includes the bands which Denburg labels F1 and F2 (Fig. 2, pattern 1). Muscles 177d' and 177e' have a pattern with the F1 and F2 bands and also the bands S1-7 (Fig. 2, pattern 2). The pattern from muscles 177d and 177e does not have the F1 and F2 bands but has bands S1-7 and an additional band, S8, which is supposed to be associated with inhibitory innervation³ (Fig. 2, pattern 3). Our gels show a number of differences from

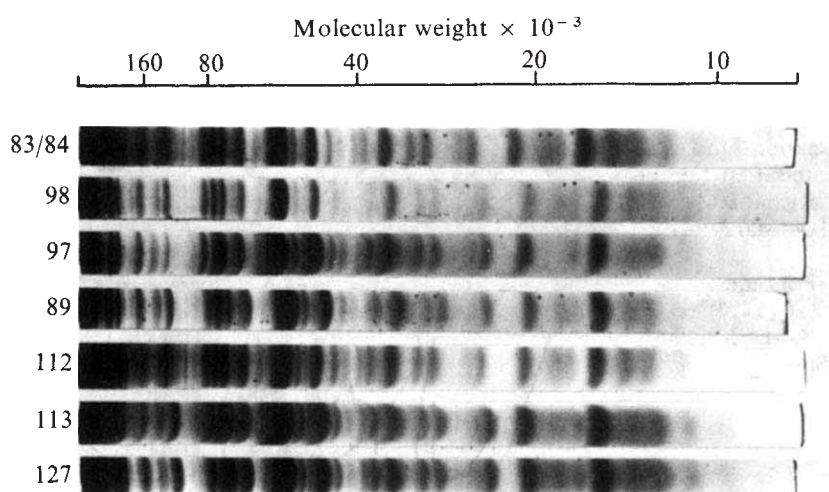


Fig. 3 SDS gels of the locust muscles shown in Fig. 1b, prepared in the same way as those in Fig. 2. The cells innervating these muscles are known¹⁰⁻¹² and are distinct, and yet the banding patterns show no distinct differences from one another. It is concluded that none of these bands can represent molecules by which ingrowing nerves could recognise specific muscles.

Denburg's, for example, an additional band labelled * (Fig. 2) but these are minor.

Analysis of other cockroach muscles (Fig. 1) shows that coxal muscles 181a, b and 182, and the thoracic muscles 174 and 176 also show pattern 1 (Fig. 2). Muscles 180 and 181c, 182c, d and 177c show pattern 2, which is also seen in the thoracic muscles 161, 163 and 167 (not illustrated). Muscle 177a shows the pattern 3 banding (Fig. 2). These results show that the characteristic patterns are not confined to those muscles innervated by cells 28 and 52 and argues against their representing specific recognition molecules. Intracellular microelectrode recording from the metathoracic coxal muscles^{6,7} and from the homologous muscles in the mesothoracic segment⁸ tends to support the alternative hypothesis that the patterns are related to 'fast' and 'slow' innervation (see Table 1). Pattern 1 muscles 181a, b are the homologues of the mesothoracic muscles 139a, b which Usherwood⁸ has shown have only 'fast' innervation. The innervation of 174 and 176 is unknown but it is predicted that these have only 'fast' innervation. Pattern 2 muscles 182c, d have 'mixed' innervation⁷, while 180 and 181c are homologous with 138 and 139c which also have 'mixed' innervation⁸. The innervation of thoracic muscles 161, 163 and 167 is unknown but it is predicted that these also have 'mixed' innervation. There are two anomalous results. Muscle 177a is a pattern 3 muscle and 177c is a pattern 2 muscle yet the mesothoracic homologues 135a and 135c have only 'fast' innervation⁸. This may reflect a true difference between the meso- and metathoracic segments, but it is more likely that the discrepancy arises from the difficulty of identifying unequivocally the subdivisions of these muscles from the accounts given in the literature^{5,9}.

Further evidence against Denburg's interpretation is provided by analysis of a group of flight muscles in the locust (Fig. 1b) in which the innervation is well known both physiologically and by intracellular filling of the motoneurons¹⁰⁻¹². Muscles 97, 113 and 127 each have one 'fast' motoneuron, muscles 83/84, 89 and 98 are innervated by the two 'fast' neurones each, and 112 by five 'fast' neurones¹⁰⁻¹². The banding patterns from extracts of these muscles are different from those in the cockroach, but almost identical with each other (Fig. 3). Each of these muscles has a distinct role in flight behaviour, and co-ordination would be impossible if a muscle were wrongly innervated. Even so, depressor muscles 97 and 127 which are immediately adjacent to elevator muscles 83/84 and 113 (Fig. 1b) have the same banding pattern. The bands detected by electrophoresis cannot, therefore, represent molecules by which

an ingrowing nerve recognises its correct muscle. It is concluded that the bands observed are characteristic of 'fast' innervated locust muscle.

We suggest that the distinctive bands detected by gel electrophoresis of whole muscle are indicative of the physiological type of the muscle rather than representing specific recognition molecules. They could be associated with either the receptor proteins or contractile mechanisms and this is worth investigating further. An immediate benefit of the results is that they demonstrate that the method may be useful for identifying the type of innervation of individual muscles.

We thank Dr Denburg for stimulating correspondence over our differences in interpretation of these results and for pointing out a discrepancy in our original interpretation of the gels for muscle 182c, d. We thank also Dr K. J. Indge and Mr G. Perry for help with electrophoresis and Drs J. S. Altman and M. D. Houslay for reading the manuscript.

N. M. TYRER
K. A. JOHNSON

Department of Biochemistry,
University of Manchester Institute of
Science and Technology, Manchester, UK

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- ¹ Young, D. in *Developmental Neurobiology of Arthropods* (ed. Young, D.) 179-202 (Cambridge University Press, London, 1973).
- ² Pearson, K. G. & Bradley, A. B. *Brain Res.* **47**, 492-496 (1972).
- ³ Denburg, J. L. *Nature* **258**, 535-537 (1975).
- ⁴ Fairbanks, G., Steck, T. L. & Wallach, D. F. H. *Biochemistry* **19**, 2606-2617 (1971).
- ⁵ Carbonell, C. S. *Smithson Misc. Collns* **107**, 1-23 (1947).
- ⁶ Pearson, K. G. & Iles, J. F. *J. exp. Biol.* **54**, 215-232 (1971).
- ⁷ Pearson, K. G. & Bergman, S. J. *J. exp. Biol.* **50**, 445-471 (1969).
- ⁸ Usherwood, P. N. R. *J. Insect Physiol.* **8**, 31-52 (1962).
- ⁹ Guthrie, D. M. & Tindall, A. R. *The Biology of the Cockroach* (Edward Arnold, London, 1968).
- ¹⁰ Bentley, D. R. *J. Insect Physiol.* **16**, 905-918 (1970).
- ¹¹ Tyrer, N. M. & Altman, J. S. *J. comp. Neurol.* **157**, 117-137 (1974).
- ¹² Burrows, M. J. *comp. Physiol.* **83**, 165-178 (1973).

Table 1 Summary of analysis of cockroach muscles (column 1), and their pattern types as determined by polyacrylamide gel electrophoresis (column 3)

Metathoracic muscle number	Equivalent mesothoracic muscle number (where relevant)	Pattern type	Type of innervation
178	—	1	Fast ⁶
179	—	1	Fast ⁶
181a, b	139a, b	1	Fast ⁸
174	—	1	Unknown
176	—	1	Unknown
177d', e'	—	2	Mixed ⁶
180+181c	138+139c	2	Mixed ⁸
182c, d	—	2	Mixed ⁷
161	—	2	Unknown
163	—	2	Unknown
167	—	2	Unknown
177c*	135c	2	Fast**
177d, e	—	3	Slow
177a*	135a	3	Fast**

The equivalent muscles in the mesothoracic segment for which the innervation is known from microelectrode studies⁸ are given in column 2. The type of innervation deduced from these and other microelectrode studies^{6,7} is given in column 4. The two anomalous results are shown by *. These may be caused by difficulty of identifying unequivocally these subdivisions of muscle 177.

Increased membrane fluidity precedes fusion of muscle cells

EMBRYONIC skeletal muscle cell cultures are highly suitable for demonstrating the dynamic role of the plasma membrane in cellular differentiation. A primary event in the overt development of muscle in culture is fusion of the plasma membranes of neighbouring mononucleated myoblasts to form multinucleated myotubes^{1,2}. Previous studies of muscle fusion have focused on ultrastructural³ and electrophysiological⁴ aspects, the requirement for Ca²⁺ (ref. 5), and the importance of fusion for the subsequent biosynthesis of muscle-specific proteins⁵. But, understanding of this process has been hampered by the absence of reliable markers for the initiation of myoblast fusion⁴. In addition, the importance of the lipid properties of the cell membrane in regulation of the onset of muscle fusion has not been unambiguously demonstrated in spite of the well-established role of lipids in other cases of membrane fusion⁶. We examined the possibility that fusion of myoblasts is regulated by changes in the lipid fluidity of the cell membrane, as measured by the rotational diffusion of a fluorescent probe. We report here that, as a primary step in myogenesis in culture, mononucleated myoblasts undergo a sharp decrease in membrane microviscosity, and shortly afterwards fuse rapidly to form multinucleated myotubes. The microviscosity remains at a minimal value during the period of rapid cell fusion. The subsequent post-fusional differentiation of the muscle cell is accompanied by the regeneration of membrane rigidity.

Primary cultures of embryonic chick breast muscle cells were prepared⁷ and plated at initial densities of 1.5-2 × 10⁶ cells per 60-mm culture dish. Cultures were grown in Dulbecco's modified Eagle's medium⁸ (88%, v/v) supplemented with horse serum (Gibco, 10%, v/v) and chick

embryo extract (2%, v/v). The extent of fusion was expressed as the fraction of nuclei located in multinucleated cells⁹.

Microviscosity was determined by monitoring the fluorescence polarisation of the lipid probe 1,6-diphenyl-1,3,5-hexatriene (DPH) as previously described^{10,11}. Fluorescence labelling of cultured muscle was carried out by incubating muscle cell suspensions in Dulbecco's phosphate-buffered saline (PBS) (ref. 12) containing 1 μ M DPH (ref. 11) for 15 min at 37 °C. Labelled cells were washed and suspended in PBS at a concentration of 0.2–0.4 mg cell protein per ml. As shown by fluorescence microscopy, the DPH-induced fluorescence was confined to the muscle cell periphery.

In the culture conditions used in this study, the differentiation of muscle cells is marked by a high degree of synchrony. Within an interval of approximately 1 d, synchronously differentiating cultures of embryonic chick myoblasts withdraw from the mitotic cycle, undergo rapid fusion, and initiate the accumulation of contractile proteins⁹ and specialised membrane components such as the acetylcholine receptor and acetylcholinesterase¹³. As shown in Fig. 1, fusion of myoblasts was initiated during the second day in culture. The proportion of fused cells increased sharply and most nuclei were located in multinucleated myotubes within several hours. During the initial day in culture and before fusion, myoblasts underwent a steep decrease in microviscosity (Fig. 1). The microviscosity reached minimal levels concomitantly with onset of myoblast fusion and remained unchanged during the main period of fusion. Shortly after the degree of fusion had reached a maximum, an increment in microviscosity could be detected. Post-fusional differentiation of myotubes was accompanied by a continuous increase in microviscosity

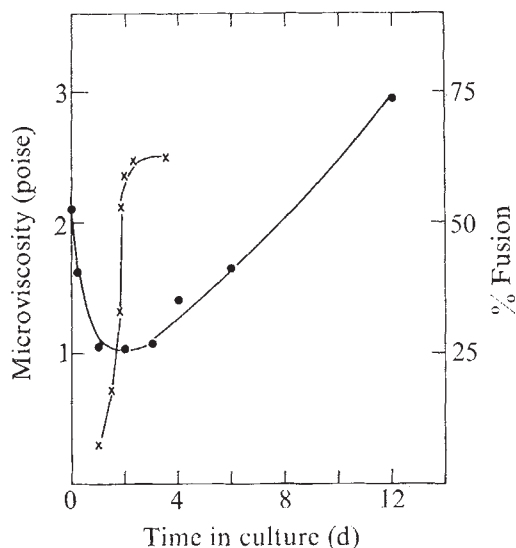


Fig. 1 Kinetics of fusion and change in microviscosity during differentiation of embryonic chick skeletal muscle in culture. Fusion (x) is expressed as the % of nuclei found in multinucleated cells. Microviscosity (●) was measured at 25 °C as before^{10,11} with muscle cells labelled with DPH as follows. Cultures were rinsed with PBS and cells were suspended by 5 min exposure to 0.2% trypsin solution (Gibco). After a rinse with 5 ml PBS containing 0.25 mg Lima bean trypsin inhibitor (Sigma), the cells were resuspended in the labelling dispersion of 1 μ M DPH in PBS and incubated at 37 °C for 15 min. The cells were collected by centrifugation at 2,000 r.p.m. for 5 min and resuspended in PBS. As an alternative procedure, the muscle cell monolayer was fluorescently labelled on the culture dish by exposure to 5 ml of the labelling dispersion for 15 min at 37 °C. The cells were rinsed with buffer, removed from the dishes as described above, rinsed and resuspended in PBS. Similar results were obtained with both labelling procedures. For convenience, cells were routinely labelled in suspension and measurements were performed with 0.2–0.4 mg cell protein per ml PBS.

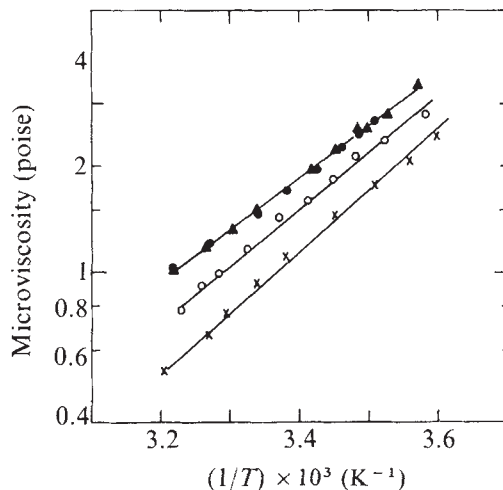


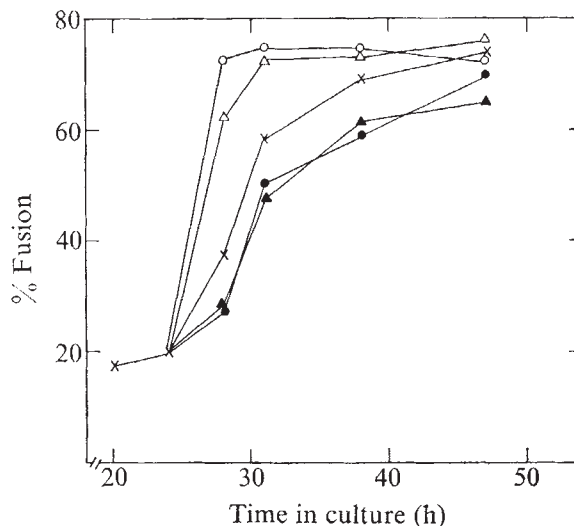
Fig. 2 Temperature dependence of microviscosity of muscle cultures at various stages of development; ●, 5 h; x, 48 h; ○, 96 h; ▲, 144 h after plating.

(see Fig. 1) coinciding with the developmental changes in biochemical¹³ and electrophysiological¹⁴ properties of the muscle cell membrane. This rise was maintained during the second week of maturation in culture to reach values higher than the initial microviscosity measured at the time of plating. Thus the pre-fusional decrease in microviscosity is a transitory event which is reversed in the course of subsequent differentiation of muscle in culture.

Figure 2 shows the dependence on temperature of microviscosity in muscle cells at various stages of maturation. As shown, the relation between microviscosity (η) and the absolute temperature (T) fits the expression $\eta = A \exp(\Delta E/RT)$ which eliminates the possibility of lipid phase transitions in the muscle cell membrane both before and after fusion¹⁵. As calculated from the plots of $\log \eta$ against $1/T$ (Fig. 2), the flow activation energy (ΔE) for all cultures tested, falls in the range of 6.5–8.0 kcal mol⁻¹, a value characteristic of cell membranes¹¹.

The marked increase in membrane fluidity observed before the fusion of myoblasts is consistent with the suggestion that high lipid fluidity is essential for fusion¹⁶.

Fig. 3 Effects on kinetics of fusion of exposure of myoblasts to fatty acids. Fusion was scored after addition of 2×10^{-5} M stearic (●), elaidic (▲), oleic (△) or linoleic (○) acids to replicate culture dishes 24 h after plating and compared with control cultures (x). The extent of fusion was expressed as the fraction of nuclei located in multinucleated cells⁹.



This notion is supported by the reported inhibitory effects of cholesterol and reduced temperature on myoblast fusion¹⁷. Moreover, we have observed that exposure of myoblasts to fatty acids that increase membrane microviscosity (stearic or elaidic acids, 2×10^{-5} M) (ref. 18) retarded fusion (Fig. 3). Conversely, treatment of cells with fatty acids that decrease membrane microviscosity (oleic or linoleic acids, 2×10^{-5} M) (ref. 18) facilitated fusion. These findings suggest the importance of cell membrane lipid properties in the regulation of muscle cell fusion and indicate that the sharp increase observed in myoblast membrane fluidity is a reliable marker for the initiation of fusion. The transient fluidisation of the myoblast membrane which allows fusion could be regulated by developmental changes in lipid composition such as an overall increase in phospholipid content or increase in the degree of unsaturation of acyl chains.

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J. PRIVES

Department of Neurobiology,

M. SHINITZKY

Department of Membrane Research,
The Weizmann Institute of Science,
Rehovot, Israel

Received 4 March; accepted 30 June 1977.

- 1 Yaffe, D. *Curr. Top. dev. Biol.* **4**, 37–77 (1969).
- 2 Fischman, D. A. in *The Structure and Function of Muscle* 2nd edn Vol. 1, 75–149 (ed. Bourne, G. H.) (Academic, New York, 1972).
- 3 Lipton, B. H. & Konigsberg, I. R. *J. Cell Biol.* **53**, 348–364 (1972).
- 4 Rash, J. E. & Fambrough, D. *Dev. Biol.* **30**, 166–186 (1973).
- 5 Shainberg, A., Yagil, G. & Yaffe, D. *Dev. Biol.* **25**, 1–29 (1971).
- 6 Poste, G. & Allison, A. C. *Biochim. biophys. Acta* **300**, 421–465 (1973).
- 7 Paterson, B. & Prives, J. *J. Cell Biol.* **59**, 241–245 (1973).
- 8 Smith, J. D., Freeman, G., Vogt, M. & Dulbecco, R. *Virology* **12**, 185–196 (1960).
- 9 Paterson, B. & Strohmman, R. C. *Dev. Biol.* **29**, 113–138 (1972).
- 10 Shinitzky, M. & Barenholz, Y. *J. biol. Chem.* **249**, 2652–2658 (1974).
- 11 Shinitzky, M. & Inbar, M. *Biochim. biophys. Acta* **433**, 133–149 (1976).
- 12 Dulbecco, R. & Vogt, M. *J. exp. Med.* **99**, 167–182 (1954).
- 13 Prives, J. M. & Paterson, B. *Proc. natn. Acad. Sci. U.S.A.* **71**, 3208–3211 (1974).
- 14 Ritchie, A. K. & Fambrough, D. M. *J. gen. Physiol.* **66**, 327–355 (1975).
- 15 Lentz, B. R., Barenholz, Y. & Thompson, T. E. *Biochemistry* **15**, 4521–4528 (1976).
- 16 Papahadjopoulos, D., Poste, G., Schaeffer, B. E. & Vail, W. J. *Biochim. biophys. Acta* **352**, 10–28 (1974).
- 17 Van Der Bosch, J., Schudt, C. & Pette, D. *Expl Cell Res.* **82**, 433–438 (1973).
- 18 Chapman, D. & Wallach, D. F. H. in *Biological Membranes* (ed. Chapman, D.) 125–202 (Academic, London, 1968).

Evidence for multispecificity of antibody molecules

ONE of the salient questions in immunology concerns the means by which the immune system is capable of recognising an apparently limitless variety of antigenic determinants, including many that presumably did not exist during its evolution. Three major hypotheses have been put forward to explain the diversity of the immune response: a stringent germ line hypothesis, a recombinational germ line hypothesis and a somatic mutation hypothesis (see ref. 1 for discussion). If the assumption is made that an antibody-combining site has a single antigenic specificity, the first of the above hypotheses postulates about 1,000 variable genes and 1,000 constant genes to code for about 10^6 antigenic determinants. The second and, especially, the third hypotheses require the presence of many fewer genes in the germ line. Even fewer genes would be required if antibody-combining sites were multispecific. Evidence has been put forward to support the concept of multispecificity². Moreover, the ability of single myeloma globulins to bind structurally diverse molecules³ and the multispecific character of a Bence-Jones⁴ dimer can be taken as additional supportive evidence. In an earlier paper⁵, we attempted

to investigate the question of multispecificity by studying the binding specificities of a population of antibodies of limited heterogeneity generated by immunisation with a conjugate of adenosine-5'-phosphate and gramicidin S: (AMP)₂-gramicidin S (ref. 6). Immunised rabbits produced predominantly three or four globulin proteins specific for AMP. Binding studies on the sera of one of the rabbits, in which there were three major AMP-specific globulins, yielded data that could be interpreted as evidence for multispecificity of antibody-combining sites. We have now succeeded in isolating three homogeneous anti-AMP antibodies from one of the rabbits immunised with (AMP)₂-gramicidin S and have studied the ability of each globulin to bind AMP and other ligands. The isoelectric pattern of the specifically purified⁷ population of antibodies obtained from the serum of rabbit 449 (ref. 5) showed three major bands. The three bands were separated and isolated by preparative isoelectric focusing in polyacrylamide by an adaption of the procedure of Wrigley⁸, and were shown to be homogeneous by analytical isoelectric focusing. They were designated fractions A, B and C.

Table 1 Inhibition of reaction between antibody and AdGase by haptens (relative concentration for 50% inhibition)*

Inhibitors	Fraction A	Fraction B	Fraction C
AMP	1 (8 nmol)†	1 (8 nmol)	1 (10 nmol)
DNP-glycine	75	8	100
DNP-lysine	75	10	>1,000
Menadione	2	16	>1,000
Hydralazine	825	26	>1,000
Caffeine	6	50	4
GMP	575	83	>1,000
Sodium barbital	>1,000	>1,000	>1,000
Procaineamide-HCl	>1,000	>1,000	>1,000
CMP	>1,000	>1,000	>1,000
UMP	>1,000	>1,000	>1,000
TMP	>1,000	>1,000	>1,000

*Twenty-five microlitres of a 1:50 dilution of each purified antibody (final concentration about $5 \mu\text{g ml}^{-1}$) in 0.85% NaCl–1% rabbit serum albumin were incubated with 25 μl inhibitor (1 nmol to 10 μmol) in 0.85% NaCl for 2 h at 25 °C. To this mixture was added 25 μl Ad Gase (ref. 9) (originally 10 mg ml⁻¹) diluted 1:10,000 in 0.1 M phosphate buffer, pH 7.0. After incubation at 4 °C overnight, 25 μl normal rabbit globulin (2 mg ml⁻¹) and 200 μl of sheep antirabbit globulin (25 mg ml⁻¹) were added and the mixture was incubated for another 2 h at 4 °C. Seven-tenths of a millilitre of 0.01 M Tris-HCl buffer, pH 7.5, containing 1% RSA, 0.01 M MgCl₂, 0.01 M NaCl and 0.01 M mercaptoethanol were added to each tube. The tubes were then centrifuged at 3,700g for 15 min at room temperature. One-hundred microlitres of the supernatant were added to a test tube containing the following mixture: 1.0 ml 0.3 M phosphate buffer, pH 7.0, containing 0.003 M MgCl₂, 0.6 ml distilled water, 0.3 ml 1 M mercaptoethanol and 1.0 ml solution of fluorogenic substrate, 4-methylumbelliferyl- β -D-galactopyranoside (Nortok Associates, Lexington, Massachusetts) taken from a solution containing 14 mg per 100 ml in 0.3 M phosphate buffer, pH 7.0. The mixture was incubated at 25 °C for 18 h. Then, 2 ml 0.5 M glycine-NaOH buffer, pH 10, were added to the assay mixture to terminate the reaction, and the fluorescence was measured in a Perkin Elmer Model MPF-2A Fluorescence Spectrophotometer (excitation 360 nm, emission 440 nm). Normal rabbit globulin (1:50 dilution) was used in place of antibody as a control.

†Figures in parenthesis indicate amount of AMP to give 50% inhibition.

The specificity of each antibody was examined by an enzyme immunoassay procedure, in which binding of adenosine- β -galactosidase⁹ (AdGase) was determined in the presence and absence of competing ligands. The results are given in Table 1. For all three fractions, AMP inhibited binding of Ad Gase best, but CMP, UMP, TMP and, except for fraction B, GMP were relatively poor inhibitors. On the other hand, fraction A cross reacted significantly with menadione, caffeine, DNP-glycine and DNP-lysine. Fraction B was the least discriminating of the antibodies, significant cross reactions being observed with six ligands. Fraction C, on the other hand, cross reacted well with only one of the ligands chosen for our studies, namely caffeine.

The results given in Table 1 were confirmed for fraction A by a

haemolytic assay with sheep red blood cells coated with adenosine (Table 2). Inhibition of haemolysis was best with AMP followed in order of decreasing effectiveness by menadione, caffeine and DNP-glycine. GMP, hydralazine, sodium barbital and procaineamide were very poor inhibitors. These results closely approximate those obtained in the enzymeimmunoassay (Table 1).

To establish with greater certainty that cross reactions were indeed occurring at the combining sites of the antibodies, a study was made of direct binding of ligand-coated red cells and subsequent lysis by complement. The results (Table 3) show that fractions A and B caused lysis of AMP-, DNP- and menadione-coated cells. Fraction C did not cause lysis of menadione-coated

Table 2 Haemolytic inhibition assays with fraction A and sheep erythrocytes coated with adenosine*

Inhibitors	Inhibition Titre†
AMP	1 nmol
Menadione	1 nmol
Caffeine	5 nmol
DNP-glycine	50 nmol
GMP	100 nmol
Hydralazine	100 nmol
Sodium barbital	100 nmol
Procaineamide-HCl	1 μ mol

*Adenosine-coated erythrocytes were prepared as follows. Adenosine (20 mg) was dissolved in 1 ml 0.1 M NaIO₄ and allowed to stand for 20 min in the dark. One drop of ethylene glycol was then added. A 5% sheep erythrocyte suspension (1 ml) in 0.01 M phosphate-0.85% NaCl, pH 7.5 (PBS), was added to the periodate solution and the pH was adjusted to 8 with 0.1 M NaOH. Thirty minutes later, 83 mg Na(H₂BCN), freshly dissolved in 1.0 ml PBS was added and the suspension was allowed to stand for another 15 min. The adenosine-coated erythrocytes were then washed twice in PBS and made up as a 0.75% (v/v) suspension in PBS. The passive haemolytic titre of fraction A was determined by microtitration; PBS, pH 7.0, was used as diluent. Aliquots (25 μ l) of serially twofold-diluted antibody samples, 25 μ l 0.75% (v/v) suspension of adenosine-coated sheep erythrocytes, 5 μ l sheep anti-rabbit globulin (diluted 1:100) and 10 μ l guinea pig complement (Pel-Freez Biologicals) (diluted 1:10 with PBS) were pipetted, in that sequence, into test tubes. After 1 h at 37 °C, the haemolytic endpoint was found to be at 1:16 dilution of antibody. For the haemolytic inhibition studies, 5 μ l various inhibitors (100 pmol to 1 μ mol) were added to 25 μ l fraction A diluted 1:8. After 1 h at room temperature, 25 μ l 0.75% adenosine-coated erythrocyte suspension, 5 μ l sheep anti-rabbit globulin (diluted 1:100 in PBS) and 10 μ l guinea pig complement (diluted 1:10 in PBS) were added. Haemolysis was allowed to proceed for 1 h at 37 °C and the titre determined.

†Lowest quantity of inhibitor for which there is still inhibition of haemolysis.

cells. These results are consistent with the findings presented in Tables 1 and 2.

Cross specificity could also be demonstrated at the surface of lymphocytes. Peripheral lymphocytes were obtained¹⁰ from rabbit 449. Their ability to bind AdGase in the presence of various ligands was determined by a modification of a published fluorometric assay^{9,10}. The results (Table 4) show that at the single concentration measured, AMP, menadione and hydralazine were essentially identical in their ability to displace AdGase from the population of lymphocytes capable of binding the latter. With respect to the other ligands, the activities were as follows: barbital > caffeine > GMP > DNP-glycine. Procaineamide and UMP were essentially inactive. The results show that receptor sites on peripheral lymphocytes are also multispecific. Generally, ligands that competed well were the same ones that did so in the experiments with circulating antibody. Exact comparisons are not valid, however, since in the lymphocyte assay one is also examining the specificities of cells that were not stimulated to produce anti-AMP antibodies by (AMP)₂-gramicidin S. We know from our previous studies⁵ that A-ovalbumin stimulated at least a hundred-fold more clones than did (AMP)₂-gramicidin S. These additional cells should also bind AdGase and have different spectra of cross-reactions.

Our data support the notion² that combining sites of antibodies

Table 3 Haemolytic assays with hapten-coated sheep erythrocytes*

Hapten	% Lysis of coated sheep erythrocytes†			
	Anti-AMP			
	Fraction A	Fraction B	Fraction C	Anti-A ovalbumin
Adenosine	100 (1:16)‡	100 (1:8)	100 (1:8)	100 (1:640)
DNP	45	81	16	100 (1:64)
Menadione	60	24	0	100 (1:32)

*DNP-erythrocytes: 2,4-dinitrobenzene sulphonic acid (sodium salt; Eastman) in 1.0 ml PBS was added to 1.0 ml 5% suspension of sheep erythrocytes in PBS. The suspension was left at room temperature with intermittent shaking for 2 h. Excess reagent was removed by washing several times with PBS. Menadione-coated erythrocytes: A saturated solution of menadione was made up in PBS. A 5% erythrocyte suspension (1 ml) was exposed to 5 ml of the saturated solution for 30 min, followed by two washings with PBS.

†Haemolytic assay carried out as described in Table 2. Quantification of haemoglobin released from lysed erythrocytes was carried out by adding 1 ml PBS to each testtube after the second incubation period. The supernatant was recovered by centrifugation at 150g for 5 min at 4 °C and haemoglobin was determined spectrophotometrically at 413 nm. Haemoglobin released by cells lysed by distilled water represented 100% lysis.

‡Numbers in parenthesis denote highest dilution of antibody that caused lysis; otherwise, antibody was undiluted and was at concentration of about 200 μ g ml⁻¹. Uncoated sheep erythrocytes + antibody and coated erythrocytes with pre-immune sera showed no lysis.

are multispecific. Thus, they share the property of other ligand-binding proteins (such as enzymes) of being able to bind more than a single ligand. Like enzymes, they can bind ligands of diverse structure, and, again, as with enzymes, effective ligands need have no obvious resemblance to the primary 'specific' ligand (see, for example, ref. 11). Antibody-combining sites can be large enough to accommodate five or six sugar residues¹². The contact surfaces, therefore, are complex. One would not expect to be able to predict the types of different structures that can be accommodated. Indeed, our results show that predictions are not feasible. Our anti-AMP antibodies do not bind GMP, UMP or TMP well. Yet, they bind such molecules as menadione and hydralazine. With respect to the former, we have carried out preliminary studies to determine the types of forces involved in its binding to fractions A and B. Surprisingly, its ability to bind decreased markedly with temperature indicating that hydrophobic forces are not predominant¹³. AMP, on the other hand, bound equally as well at 0 °C and at 37 °C, suggesting that hydrogen bonds, electrostatic forces and hydrophobic forces might all be involved in its binding to specific antibody.

Although we think it likely, our data cannot be used to show that different subsites of the combining site are involved in the binding of various ligands. We would like to establish this with certainty by attempting to isolate sufficient antibody protein to attempt

Table 4 Inhibition of binding of AdGase to lymphocyte surface receptors by various inhibitors

Inhibitors (20 nmol)	% Inhibition†
AMP	28 ± 3
Menadione	31 ± 3
Hydralazine	28 ± 3
Sodium barbital	20 ± 2
Caffeine	12 ± 1
GMP	7 ± 1
DNP-glycine	2 ± 1
Procaineamide	0
UMP	0

The assay procedure was a modification of one described previously¹⁰. Peripheral blood lymphocytes (2×10^6 in 50 μ l PBS) were incubated with 20 nmol ligand in 25 μ l PBS at 4 °C. After 90 min, 50 μ l of a 1:1000 dilution of AdGase were added and incubation was continued at 4 °C for another 3 h. PBS (2 ml) was added to each sample and the cells were collected on 25-mm diameter Whatman No. 1 filters placed in a Millipore manifold. A 1:1000 dilution of AdGase (50 μ l), without cells, was also filtered through the paper to serve as a blank. All filters were then washed with 20 ml PBS and placed in test tubes containing the assay solution described previously (footnote to Table 1) and incubated for 30 min at 25 °C in a shaking water bath. All subsequent procedure described previously¹⁰.

†Given as mean $\pm$ s.d. mean of four experiments done in duplicate.

crystallisation and X-ray crystallographic examination. This is not necessarily a trivial point since it would definitely rule out the possibility that all competing ligands resemble each other sufficiently to explain the crossreactions. For example, Michaelides and Eisen¹⁴ used this argument to explain cross reaction between menadione and DNP in myeloma proteins. This is a very difficult argument to dispose of, since interactions between ligands and binding proteins take place mainly by way of functional groups and/or hydrophobic surfaces. There are only a limited number of the former, and very many ligands have hydrophobic areas. Thus, we can always find structural resemblances, particularly with small ligands. Each of our three purified AMP-binding proteins, however, has its own cross specificities, a finding that would not have been expected if there were thermodynamically important structural similarities among the ligands. The finding that each of the anti-AMP proteins bound a different spectrum of other ligands is in agreement with the proposal of Richards *et al.*² that the high specificity of the immune response lies not only in the properties of a single antibody molecule but is due also to the production of a heterogeneous population of antibody molecules all sharing specificity for AMP (as we found with A-ovalbumin as an immunogen)⁵ but with each member having its own spectrum of crossspecificities.

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DEBORAH J. CAMERON

BERNARD F. ERLANGER

Columbia University,
Department of Microbiology,
New York, New York 10032

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- ¹ Leder, P. *et al.* in *Molecular Approaches to Immunology* (eds Smith, E. E. & Ribbons, D. W.) Miami Winter Symposium 9, 173 (Academic, London and New York, 1975).
- ² Richards, F. F., Konigsberg, W. H., Rosenstein, R. W. & Varga, J. M. *Science* **187**, 130 (1975).
- ³ Rosenstein, R. W., Musson, R. A., Armstrong, M., Konigsberg, W. H. & Richards, F. F. *Proc. natn. Acad. Sci. U.S.A.* **69**, 877 (1972).
- ⁴ Edmundson, A. B. *et al.* *Biochemistry* **18**, 3816 (1974).
- ⁵ Cameron, D. J. & Erlanger, B. F. *Immunochimistry* **13**, 263 (1976).
- ⁶ Montgomery, P. C., Rockey, J. H. & Williamson, A. R. *Proc. natn. Acad. Sci. U.S.A.* **69**, 228 (1972).
- ⁷ Szafran, H., Beiser, S. M. & Erlanger, B. F. *J. Immun.* **108**, 1157 (1969).
- ⁸ Wrigley, C. W. *J. Chromatog.* **36**, 362 (1968).
- ⁹ Lauer, R. C. & Erlanger, B. F. *Immunochimistry* **11**, 533 (1974).
- ¹⁰ Cameron, D. J. & Erlanger, B. F. *J. Immun.* **116**, 1313 (1976).
- ¹¹ Bernhard, S. A., Lee, B. F. & Tashjian, Z. H. *J. molec. Biol.* **18**, 405 (1966).
- ¹² Kabat, E. A. *J. Immun.* **97**, 1 (1966).
- ¹³ Scheraga, H., Nemethy, G. & Steinberg, I. Z. *J. biol. Chem.* **237**, 2506 (1962).
- ¹⁴ Michaelides, M. C. & Eisen, H. N. *J. exp. Med.* **140**, 687 (1974).

of the IgG lobes is given by Padlan¹⁰. One of the fundamental questions involved in antibody function is the nature of the mechanism by which binding of antigen to the Fab regions affects the Fc region. Huber *et al.* have recently outlined a possible allosteric model for the activation step on the basis of X-ray and other data⁹. In this note we use simple diffusion arguments to estimate the characteristic times associated with the Huber proposal. Since the analysis deals only with the relative motion of the IgG domains, it is possible that slower intradomain structural changes are also involved in the activation process; if so, the estimated characteristic times would be lower bounds for the activation time. The role of conformational transitions in effector function activation is still in dispute¹¹, but we note that the diffusional model may be useful for analysing other dynamical phenomena in immunoglobulins, such as their fluorescence depolarisation behaviour¹²⁻¹⁴.

Huber *et al.*⁹ assume that the lobes have relatively little interaction with each other in the unliganded IgG. Thus, neighbouring lobes are free to rotate relative to each other over certain angular ranges. Inspection of the Fab regions in the IgG structure suggests that the V_L-V_H dimer (the antigen-binding lobe) can rotate about an axis passing through the 'switch peptides' which connect it to the C_L-C_H1 dimer (the second lobe in Fab); this motion has been termed 'elbow bending'. The Fab region itself can rotate relative to the Fc region by a 'hinge bending' deformation of the peptide which links the C_L-C_H1 dimer to C_H2-C_H2, the first lobe of the Fc region. Although the 'elbow' and 'hinge' bending motions are the only ones that concern us here, it should be noted that there may also be a flexible link between the two Fc lobes.

The binding of antigen is postulated to trigger the successive 'locking' of some of these bending motions⁹. In particular, it is suggested that antigen binding induces a conformational transition in the V_L-V_H dimer that causes it to interact more strongly with the C_L-C_H1 dimer. This interaction is assumed to induce a conformational transition in the C_L-C_H1 dimer that alters its affinity for the C_H2-C_H2 dimer. The resulting coupling between the Fab and Fc regions is presumed to lead to the activation of Fc.

The relative displacements of the various domains in going from the open (unliganded) to the locked (liganded) structure can be characterised as bending motions. We assume in what follows that these bending motions can be described by a one-dimensional diffusion equation. The justification for this assumption comes from a recent theoretical study of the hinge bending mode in lysozyme¹⁵. It was found there that in spite of the forces due to the interactions between the two lobes (covalent plus non-bonded), their relative motion is diffusive in character; that is, the hydrodynamic frictional forces dominate the motion. Frictional effects are likely to be even more important in the relative motion of the IgG lobes, which are larger than those in lysozyme and apparently interact more weakly.

The one-dimensional diffusion equation is

$$\frac{\partial p}{\partial t} = D \frac{\partial^2 p}{\partial \theta^2}$$

where $p(\theta, t)$ is the probability that the elbow or hinge angle is θ at time t and D is the angular diffusion constant appropriate to the particular motion. The boundary conditions are $p(0, t) = 0$, representing locking at the angle $\theta = 0$, and $\partial p / \partial \theta = 0$ at $\theta = \theta_r$, corresponding to reversal of the bending motion at the maximum excursion angle, θ_r . If all bending angles between 0 and θ_r are equally populated initially, the average time required to reach $\theta = 0$ is $\tau = \theta_r^2 / 3D$ (ref. 16); in the limiting case that all molecules have $\theta = \theta_r$ initially, the average time becomes $\tau = \theta_r^2 / 2D$.

To obtain approximate values for the angular diffusion constants, we model the domains involved by spheres of radius a and treat the bending motions as relative rotations about an axis located a distance r from the sphere centres. The effect of the

Internal motions of antibody molecules

ANTIBODY molecules of the IgG class are composed of three covalently linked regions^{1,2}. Two of these (designated Fab) are identical and bind antigen; the third (Fc) has been identified as the site of antibody effector functions (for example, complement fixation), which are activated by antigen binding³. There is evidence that the binding of antigen to the Fab region induces an allosteric transition in IgG and that this transition may be a step in the effector activation process⁴. The findings of Pecht *et al.*⁵ indicate that for complement fixation both Fab regions must bind antigen, even though binding to one induces some conformational change in Fc. Binding of hapten does not lead to complement fixation in general, but some exceptions to this rule are known⁶. The interchain disulphide bridges must be intact for Fab ligand binding to have a productive effect on the Fc region^{7,8}. Both the Fab and Fc regions have a domain structure; each consists of a pair of compact lobes covalently linked by strands of polypeptide chains⁹. A schematic diagram of the connectivity

fluid environment is represented by using the Stokes law for the frictional coefficient of the spheres¹⁷; the correction factors for relative motion and hydrodynamic interaction approximately cancel for rough spheres which are close together¹⁸. The calculated diffusion constants in water at 300 K ($\eta = 1 \times 10^{-2}$ P) are $D_e \approx 2.8 \times 10^7$ s⁻¹ for the elbow bending ($a \approx r \approx 20$ Å) and $D_h \approx 3.4 \times 10^6$ s⁻¹ for the hinge bending ($a \approx r \approx 40$ Å).

To complete the estimate of the average times required for locking, we need to know the ranges of angular excursion, θ_r . If we use the angular differences between the 'minimum disorder' unliganded IgG structure and the hypothetical liganded structure proposed by Huber *et al.*⁹, they are $\theta_r \approx 0.7$ rad (40°) for the elbow, and $\theta_r \approx 0.5$ rad (30°) for the hinge. The resulting average time for elbow locking is $\tau_e \approx 6 \times 10^{-9}$ s and that for hinge locking is $\tau_h \approx 3 \times 10^{-8}$ s.

It is possible to test the diffusion constant values by using them to analyse fluorescence depolarisation results for antibody molecules^{12,13}. We consider a large molecule with isotropic rotational diffusion constant D_{IG} to which a labelled internal rotor with uniaxial rotational diffusion constant D_h is attached. If the angular range of internal rotation were unrestricted, the fluorescence anisotropy of the label would be a sum of exponentials, each decaying with a certain characteristic time; the characteristic time for the fastest decaying term would be $\phi_h = 1/(6D_{IG} + 4D_h)$ (ref. 19). This expression should also provide a useful estimate of the decay time due to Fab motion in IgG, although the range of internal motion is somewhat restricted in this case. Taking $6D_{IG} = 1/(1.7 \times 10^{-7}$ s) from the work of Yguerabide *et al.*¹² yields $\phi_h = 5.1 \times 10^{-8}$ s, which is near the shortest decay times measured for antibodies labelled with fluorescent haptens (3.3×10^{-8} s) (ref. 12). Thus, these results indicate either that Fab-Fc locking is somewhat less rigid in liganded antibody than supposed by Huber *et al.* or that hapten is less effective than antigen in inducing the Fab-Fc locking; circular polarised fluorescence studies support the latter possibility⁵. It is worth noting that the shortest decay times measured in similar studies using nonspecific, covalently bound labels are somewhat smaller (10^{-8} s); (ref. 13) this corresponds to the estimated shortest decay time for a labelled V_L - V_H dimer of a completely open IgG: $\phi_e \approx 1/(6D_{IG} + 4D_h + 4D_e) \approx 8 \times 10^{-9}$ s. This suggests that it is not necessary to invoke independent rotation of the fluorophore to explain the short relaxation time measured by Wahl¹³. The above results, taken together, lead to the interpretation that specific binding of hapten may reduce motion about the elbow without a comparable effect on motion about the hinge, while nonspecific attachment of labels has little effect on either motion. Thus, the fluorescence depolarisation studies are consistent with a hydrodynamic model of IgG internal motions.

A number of possible refinements of the model calculations should be considered. Although the times involved in the relaxation phenomena and the locking reaction are not expected to be very sensitive to the detailed form of the elbow and hinge bending potentials (which we have assumed to be constant in the region $0 \leq \theta \leq \theta_r$ and infinite outside), the locking times do depend quadratically on the allowed ranges of motion. In particular, the hinge bending motion could involve a larger angular range in the unliganded antibody than assumed above. This would lead to a longer average locking time; a reasonable upper estimate for θ_r ($\theta_r = 90^\circ$) gives $\tau_h \approx 2.5 \times 10^{-7}$ s. Another factor to be considered is that the diffusion constants D_e and D_h would be decreased by the binding of large antigens to the IgG. If the bending motions were to depart somewhat from the assumed one-dimensional rotational character, the conclusions of this work would not be altered significantly. If the probability of locking were less than unity at $\theta = 0$, the required correction factor could be introduced by using a partially absorbing barrier²⁰. Finally, the detailed character of the bending potentials and their role in the bending motion and the locking reaction are of considerable interest.

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J. A. MCCAMMON
M. KARPLUS

Department of Chemistry,
Harvard University, Cambridge,
Massachusetts 02138

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- Porter, R. R. *Biochem. J.* **73**, 119–126 (1959).
- Edelman, G. M. *et al. Proc. natn. Acad. Sci. U.S.A.* **63**, 78–85 (1969).
- Beale, D. & Feinstein, A. *Q. Rev. Biophys.* **9**, 135–180 (1976).
- Givol, D. in *Receptors and Recognition A* (eds Cuatrecasas, P. & Greaves, M. F.) 27–29 (Halsted, New York, 1976).
- Pecht, I., Ehrenberg, B., Calef, E. & Arnon, R. *Biochem. biophys. Res. Commun.* **74**, 1302–1310 (1977).
- Goers, J. W., Schumaker, V. N., Glovsky, M. M., Rebek, J. & Müller-Eberhard, H. J. *J. biol. Chem.* **250**, 4918–4925 (1975).
- Schur, P. H. & Christian, G. D. *J. exp. Med.* **120**, 531–545 (1964).
- Schlessinger, J., Steinberg, I. Z., Givol, D., Hochman, J. & Pecht, I. *Proc. natn. Acad. Sci. U.S.A.* **72**, 2775–2779 (1975).
- Huber, R., Deisenhofer, J., Colman, P. M., Matsushima, M. & Palm, W. *Nature* **264**, 415–420 (1976).
- Padlan, E. A. *Q. Rev. Biophys.* **10**, 35–65 (1977).
- Metzger, H. *Adv. Immun.* **18**, 169–203 (1974).
- Yguerabide, J., Epstein, H. F. & Stryer, L. *J. molec. Biol.* **51**, 573–590 (1970).
- Wahl, P. *Biochim. biophys. Acta* **175**, 55–64 (1969).
- Holowka, D. A. & Cathou, R. E. *Biochemistry* **15**, 3379–3390 (1976).
- McCammon, J. A., Gelin, B. R., Karplus, M. & Wolynes, P. G. *Nature* **262**, 325–326 (1976).
- Adam, G. & Delbrück, M. in *Structural Chemistry and Molecular Biology* (eds Rich, A. & Davidson, N.) 198–215 (Freeman, San Francisco, 1968).
- McCammon, J. A. & Wolynes, P. G. *J. chem. Phys.* **66**, 1452–1456 (1977).
- Wolynes, P. G. & McCammon, J. A. *Macromolecules* **10**, 86–87 (1977).
- Wallach, D. *J. chem. Phys.* **47**, 5258–5268 (1967).
- Karplus, M. & Weaver, D. *Nature* **260**, 404–406 (1976).

Are late replicating regions in polytene chromosomes of *Drosophila* enriched by repeated nucleotide sequences?

THE ordered highly coordinated pattern of replication of different bands is characteristic of salivary gland chromosomes of *Drosophila*^{1,2}. During the initial stage of polytene chromosome replication labelled precursor is found over Puff/interband sections while prominent bands incorporate label later and need in general a longer time for DNA synthesis^{3,4}. It is significant that regions of ectopic pairing in the chromosomes of *D. melanogaster* are of the late replicating type. We report here that late replicating DNA from *Drosophila* salivary gland cells reassociates after denaturation more rapidly than the bulk of the DNA from the same cells. A high level of repeated nucleotide sequences may be involved.

Several autoradiographic investigations have shown that there is no strict correspondence between DNA content and replication time of chromosome bands^{5,6}. In other words, it is not possible to explain the difference in the timing and duration of replication of regions studied based only on different DNA content. Thus there are at least two possibilities to explain the prolonged S-phase characteristic for definite regions in the genome of *Drosophila*:

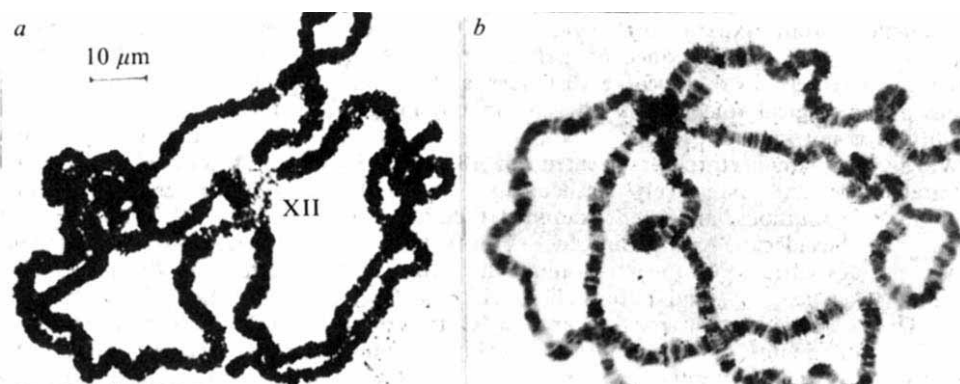
(1) The macromolecular configuration of DNA packed into chromomeres in different parts of the genome determines the number of initiation points and consequently the time necessary for the replication of a given band. Thus regions of prolonged replication may have fewer initiation sites in their DNA.

Table 1 Frequencies (%) of different labelling types at the end of third instar in *D. virilis* larvae

Time from the beginning of third instar (h)	70	72	74*
Total no. of nuclei studied	1,064	791	584
No. of labelled nuclei	284	164	96
Frequency of continuous replication pattern (%)	10.6	2.4	2.1
Frequency of discontinuous replication pattern 89.4 (final stages of S-phase) (%)	89.4	97.6	97.9

* Larvae with dark-brown anterior spiracle stage used in this investigation.

Fig. 1 *a*, Polytene chromosomes of *D. virilis* after long-term labelling; (first series) (bar represents 10 μ m); *b*, polytene chromosomes of *D. virilis* after short-term labelling. Only prominent late replicating bands incorporate 3 H-thymidine.



(2) Late replicating regions (bands) of polytene chromosomes may be enriched by repeated nucleotide sequences. This possibility comes to mind because C-heterochromatin consisting of highly repetitive satellite DNA (sDNA) is a late replicating fraction of the genome in various organisms.

To examine the latter possibility we studied renaturation kinetics of salivary gland DNA of *D. virilis* labelled by

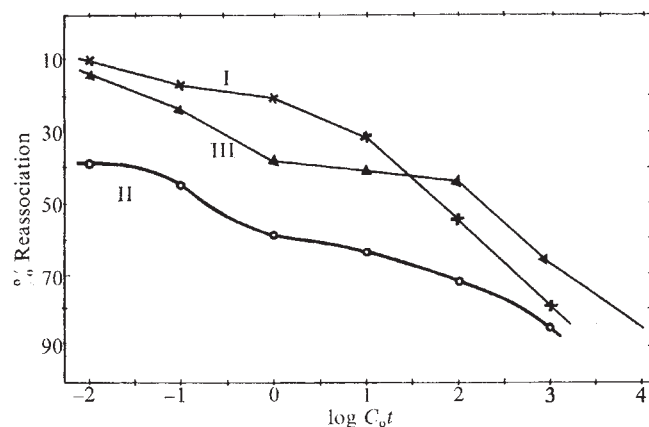


Fig. 2 Renaturation kinetics of *Drosophila virilis* salivary gland DNA labelled by different methods (I, II) and mouse DNA used as standard (III) as measured by hydroxylapatite chromatography. Samples of sheared labelled DNA were sealed in glass capillary tubes, heated to 105 °C for 10 min to denature the DNAs and then incubated at 60 °C to allow renaturation. At various times samples were removed, diluted into 1 ml of 0.12 M PB and frozen for subsequent hydroxylapatite fractionation. Single-stranded and double-stranded DNAs were eluted with ten times the bed volume of 0.12 and 0.5 PB, respectively. Renaturation was measured by 3 H-radioactivity of the hydroxylapatite fractions. I, *D. virilis* salivary gland DNA-long-term labelling (X). II, *D. virilis* salivary gland DNA-short-term labelling (O). III, Mouse DNA (standard) (Δ).

different methods. A long-term labelling was carried out in the first series of experiments. The flies were reared from oviposition to the end of third instar larvae on a medium containing 3 H-thymidine (100 μ Ci ml $^{-1}$, specific activity 4.4 Ci mmol $^{-1}$). The salivary gland chromosome DNA of such individuals undergoes several replication cycles in the presence of labelled precursor and is thus uniformly labelled (Fig. 1a). In a second series of experiments (short-term labelling) the salivary glands of third instar larvae (dark-brown anterior spiracle stage) were incubated for 15 min in a drop of Ringer insect medium supplemented with 3 H-thymidine (100 μ Ci ml $^{-1}$, 49 Ci mmol $^{-1}$).

In preliminary experiments, after short-term labelling of salivary glands from these larvae, 98% of labelled nuclei showed discontinuous labelling patterns characteristic of

end phases of polytene chromosome replication (Table 1). Thus preferential labelling of DNA of late replicating regions (bands) occurred in these experiments.

Discontinuously labelled chromosomes of the final stage of the S phase are shown in Fig. 1b. DNA from salivary glands was isolated using the cetavlon procedure⁷. DNA was sonicated and dissolved in 0.12 M Na-phosphate buffer (PB). The average fragment size of the sonicated material is about 300–400 nucleotides.

The reassociation kinetics of DNA from both series of experiments were studied using hydroxylapatite chromatography. Comparison of C_0t curves of both DNA preparations (I, II series) shows that late replicating DNA (series II) reassociates at a faster rate than the bulk DNA (series I) and hence contains significantly more repeated sequences than the genome as a whole.

The difference observed could not be due to the late replication of sDNA which does not replicate during polytenisation or at least does not replicate as frequently as euchromatic sequences⁸.

The high value of reassociation at $C_0t=10^{-2}$ in the case of late replicating DNA (series II) can not be due to snap back of palindromic sequences. Zero-time reassociation in series II equals 7%, compared with 3.8% in series I.

MICHAEL EVGEN'EV
ANDREI LEVINE

*Institute of Molecular Biology,
Academy of Sciences of the USSR,
Vavilov Str. 32, Moscow*

IRINE GUBENKO

*Institute of Cytology and Genetics,
Siberian Branch of the Academy of Sciences
of the USSR,
Prospekt Nauki 10, Novosibirsk, USSR*

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- ¹ Plaut, W., Nash, D. & Fanning, T. *J. molec. Biol.* **16**, 85–93 (1966).
- ² Kalish, W. E. & Hagele, K. *Chromosoma* **44**, 265–284 (1973).
- ³ Hagele, K. *Chromosoma* **31**, 91–198 (1970).
- ⁴ Kalish, W. E. & Hagele, K. *Chromosoma* **57**, 19–24 (1976).
- ⁵ Ananiev, E. V. & Barskii, V. E. *J. molec. Biol. (USSR)* **9**, 752–760 (1975).
- ⁶ Hagele, K. *Chromosoma* **55**, 253–258 (1976).
- ⁷ Naktinis, V. I., Malejeva, N. E., Saniko, D. F. & Mirzabekov, A. D. (submitted to *Analyt. Biochem.*)
- ⁸ Gall, J. L., Cohen, E. H. & Polan, M. L. *Chromosoma* **33**, 319–344 (1971).

Long-range attraction between red cells and a hydrocarbon surface

WE present here experimental evidence that adhesion of red cells to an oil–water interface is mediated by an attractive force acting at a distance apparently exceeding 100 nm. Although long-range forces have recently been measured between bilayer lipid lamellae in water¹ and attraction between cells has been inferred from aggregation studies^{2,3}, the long-range nature of attraction in cell

studies has previously been an assumption rather than a deduction from experimental evidence. Our analysis strongly implicates a long range attractive force, but this force is extremely weak at large distances and whether it has any biological role in cell adhesion in physiological conditions is not known.

We used human erythrocytes treated for 18 h in 3.3% glutaraldehyde and extensively washed, to prevent lysis in dilute salt solutions, and then examined their adhesion to a flat hexadecane-saline interface, formed in an apparatus described previously⁴. Water was four times distilled, twice in glass, and pure sodium chloride (a gift from Dr J. Mingins) was roasted at 700 °C to remove organic contaminants. The final pH of working solutions was 5.4 ± 0.1 . The ζ -potentials of cells and sonicated oil droplets in saline were measured in a Zeiss cytopherometer modified for low constant currents. Surface tension of method in a thermostatically controlled apparatus (± 0.1 °C). Adhesion was assessed by allowing cells to sediment under gravity in salt solution onto the oil-water interface for 20 min, then inverting the whole system (oil uppermost). The number of adherent cells was recorded photographically, the total initial number counted at the interface being greater than 400 in every case. Results were unaffected by increasing settling time to 1 h before photography.

From 145-mM NaCl down to 1.0 mM, cells remained attached to the interface indefinitely after inversion; below 1.0 mM, cells fell away from the interface (see Fig. 1). In another series of experiments cells were allowed to attach to the interface either at 10 mM or at 0.4-mM NaCl, then the system was inverted and the saline was carefully exchanged for distilled water. Between 70% and 90% of the cells fell off, whereas none lost their adhesion in a control where exchange was done with 0.4-mM NaCl. Since the control excludes mechanical removal we conclude that most of the cells in dilute saline are reversibly attached. We tested for contamination of the oil-water interface

by adherent cells by measuring the interfacial tension of a drop of 10 mM or 150 mM saline formed under hexadecane. When the saline contained cells which were allowed to settle onto the interface for 20 min the surface tension remained the same as for a drop lacking cells, to within our experimental accuracy of 0.1 dyn cm^{-1} . Red cells untreated with glutaraldehyde caused a rapid large fall in interfacial tension. We conclude that glutaraldehyde-treated cells did not contaminate the interface.

In 0.3-mM NaCl where half the red cells fall off the interface, the interface has a potential of about -30 mV and the cells -95 mV . By modelling the biconcave red cell as a torus we calculate the electrostatic repulsion for a cell interacting *en face* with the interface (as most are seen to do). We combine the computational procedures of Alamov⁵ and Brenner and Parsegian⁶ using the full non-linear Poisson-Boltzmann (PB) equation in the region where potentials exceed 25 mV . The validity of the PB equation for force calculations has recently been verified in two ways^{7,8}. We take⁹ the smaller toroidal diameter as the red cell thickness ($\sim 1.5 \mu\text{m}$), the length of the toroidal axis or Pappus line as $17 \mu\text{m}$, the density in water as 0.096 g ml^{-1} and red cell volume as $85 \mu\text{m}^3$. Such an average cell would experience a gravitational force of $8 \times 10^{-9} \text{ dyn}$. This is the size of the calculated electrostatic repulsion in 0.3-mM NaCl for a cell to interface separation of about 240 nm , that is, conditions in which an average cell is about to lose its adhesion. Thus a cell sedimenting down onto the oil-water interface under gravity alone cannot approach closer than 240 nm . This conclusion is the crux of our argument. Without an attractive force, cells would balance at 240 nm and fall off on inversion. Because half the population do not, an attractive force must reach out to at least 240 nm . To take account of the population spread, the calculation was done for conditions corresponding to 20% and 80% adhesion, giving estimates of the range of attractive force that can act at such distances is the van der Waals or electrodynamic charge fluctuation force.

Whether adhesion in dilute salt occurs at a finite separation as a result of a balance of long-range attraction and electrostatic repulsion or takes place at 'molecular contact' is not directly ascertainable from our measurement. Reversibility of adhesion is consistent with the action of long-range attractive forces. In the absence of a mathematical expression for electrodynamic attraction between a torus (or cylinder) and a plane wall which is valid for large separations where retardation will be important, one can only say that the attractive force must decrease with increasing distance. From what is known of the strength of van der Waals forces (1) (7) (10) (11) it seems virtually impossible that an electrodynamic force could pull cells in 0.3-mM NaCl into molecular contact with the interface; a force balance at a finite separation seems inescapable. This conclusion is supported by interferometric observations (in progress) on cells adherent to the interface in 0.4-mM NaCl. Adherent cells can oscillate in a direction perpendicular to the interface while remaining separated from it by a finite gap. This behaviour is not consistent with molecular contact but is precisely what would be expected from a long-range force balance.

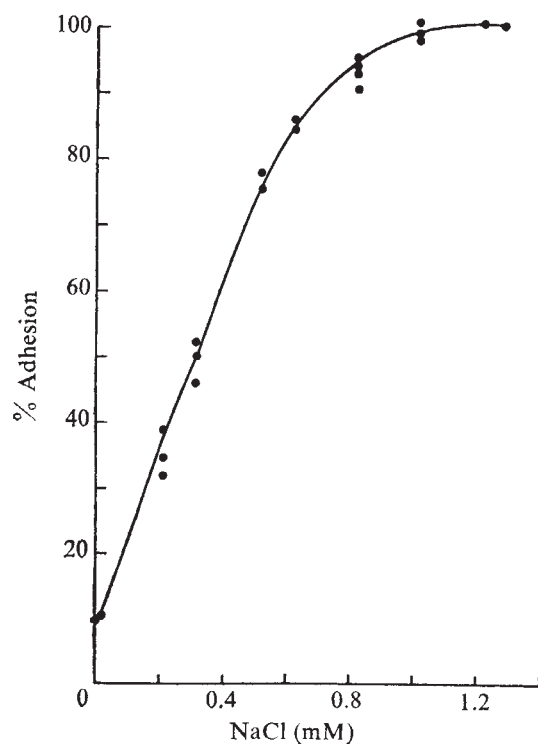
Detection of long-range attraction is of considerable interest from a physical viewpoint, but its biological significance remains uncertain.

D. GINGELL

I. TODD

Department of Biology as Applied to Medicine,
Middlesex Hospital Medical School,
Cleveland Street, London W1, UK

Fig. 1 Adhesion of red cells to hydrocarbon as a function of salt concentration.



V. A. PARSEGIAN

Division of Computer Research
and Technology,
National Institutes of Health,
Bethesda, Maryland 20014

Received 18 February; accepted 1 June 1977.

- ¹ Le Neveu, D. M., Rand, R. P. & Parsegian, V. A. *Nature* **259**, 601–603 (1976)
- ² Wilkins, D. J., Ottewill, R. H. & Bangham, A. D. *J. theor. Biol.* **2**, 176–191 (1962).
- ³ Curtis, A. S. G. *J. Embryol. exp. Morph.* **22**, 305–325 (1969).
- ⁴ Gingell, D. & Todd, I. *J. Cell Sci.* **8**, 227–239 (1975).
- ⁵ Alamo, Y. I. *Kolloidnyi Zhurnal*, **25**, 327 (1963); Engl. trans. *Colloid J. (USSR)* **25**, 313–316 (1963).
- ⁶ Brenner, S. L. & Parsegian, V. A. *Biophys. J.* **14**, 327–334 (1974).
- ⁷ Israelachvili, J. N. & Adams, G. E. *Nature* **262**, 774–776 (1976).
- ⁸ Cowley, S., Fuller, N., Rand, R. P. & Parsegian, V. A. *Biophys. J.* **17** (2) Suppl. (1977).
- ⁹ *Geigy Scientific Tables* (7th edn) (CIBA—Geigy, Basle, 1970).
- ¹⁰ Israelachvili, J. N. & Tabor, D. *Prog. Surf. Memb. Sci.* **7**, 1 (1973).
- ¹¹ Parsegian, V. A. in *Physical Chemistry: Enriching Topics from Colloid and Surface Science* (eds van Olphen, H. & Mysels, K. J.) Ch. 4 (Theorex, La Jolla, California, 1975).

The number of turns in globular proteins

PEPTIDE chain turns are those parts of a globular protein where the backbone is folded back upon itself. We show here that the number of turns is a linear function of the number of amino acid residues in the protein, and we compare the two contrasting models for turn formation. In a sequence-dependent model, turns are a linear function of the molecular weight, while in a shape-dependent model, they are a function of the two-thirds power of the molecular weight. But, a shape-dependent model also behaves, to a good approximation, as a linear function within the domain of interest. We suggest some consequences of this work for protein folding.

Peptide chain turns were first identified by Kuntz¹ and by Lewis *et al.*². Kuntz points out that turns are important for two reasons—they constitute recognisable structural units in proteins, and they are situated at the solvent accessible surface of the molecule. Rose and Seltzer³ devised an algorithm to identify peptide chain turns from coordinate data. This algorithm treats the polymer chain as a curve in space and computes a discrete radius of curvature for that curve. A turn corresponds to a locus where the chain direction vector is changing rapidly and the value of the radius of curvature is at a local minimum. Although turns can be shown to correspond to local minima in the radius of curvature, the correspondence is not 1:1; that is, some minima are not associated with turns, and additional analysis is developed to identify these loci. α -Carbon coordinate data are the only information required by the algorithm, and notions about hydrogen bonding at turn loci are irrelevant to the geometric nature of the procedure. In this sense, the algorithm provides an objective criterion for the recognition of turns as strictly structural components in proteins.

We have used the algorithm for turns to discover the number of turns in each of 21 proteins ranging in size from 53 to 450 amino acid residues (Table 1). A least squares analysis shows that the number of turns (T) is a linear function of the number of amino acid residues (R) as given by

$$T = 0.125R + 2.28 \quad (1)$$

The correlation coefficient is 0.983. An equivalent correlation exists between T and the molecular weight of the protein. These data are shown graphically in Fig. 1. The analysis is especially compelling in view of the strictly structural nature of the procedure.

We have previously defined a structural segment as the three-dimensional structure assumed by a continuous sequence of linear chain neighbours bounded by consecutive chain turns³. Clearly identification of the turns also results in identification of the structural segments, with the number of segments always

one greater than the number of turns. A structural segment, by this definition, is similar to Chothia's definition⁴ of pieces of secondary structure. Thus, the number of structural segments (S) is also a linear function of the number of amino acids as given by

$$S = 0.125R + 3.28 \quad (2)$$

Some discussion of the linear nature of the function relating turns to the number of residues is in order. In particular, a class of functions of the form

$$T = aR^n + b \quad (3)$$

can be studied where, of course, $n = 1$ when the relationship is a linear one. A choice of $n = 2/3$, however, is appropriate to the geometric argument proposed by Kuntz¹, and it turns out that the data in Table 1 will fit such a model equally well. Indeed, given suitable coefficients a and b , models of the form given in equation (3) seem to be quite insensitive to a choice of n over the domain 0.5–1.5.

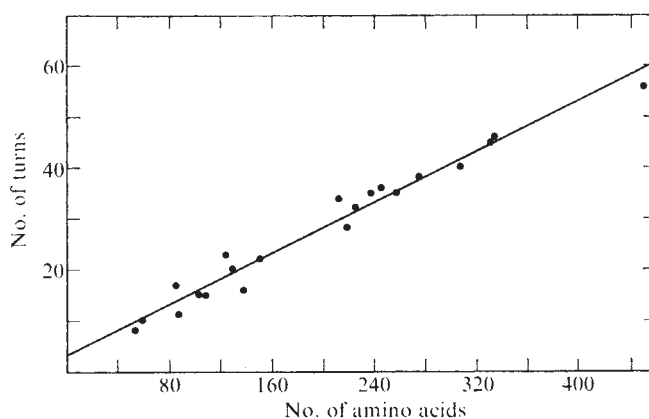


Fig. 1 A plot of no. of turns against no. of amino acids for 21 proteins. The least squares line through these points has slope 0.1245. Detailed data are given in Table 1.

To resolve the value of n , a log-log logarithmic plot of turns against number of residues was constructed. The slope of the least-squares line to these data is 0.94, much closer to linearity than to $2/3$.

Two distinct models for turn formation emerge from the above discussion. A sequence-dependent model results if turns are determined by linearly local sequences of amino acids along the polypeptide chain. For a given protein with k sites for turns imbedded within the amino acid sequence, the fraction of total residues in turns would be k/R . The data in Table 1 show that k/R is reasonably constant over a large and diverse sample of globular proteins, a fact that can be attributed to the characteristic composition of globular proteins in general¹⁰. For this reason, the data points of Table 1 cluster well around a line with a slope that is the best-fit to the values of k/R as shown in Fig. 1. The sequence-dependent model is also consistent with the success of empirical correlations between sequence and structure¹¹. The model is inherently linear, with $n = 1$ in equation (3).

In contrast, a shape-dependent model would result if turns were distributed over the globular surface of the folded protein, without particular regard for sequence. Janin¹² and Teller¹³ have shown that the surface area of proteins is proportional to the molecular weight raised to the two-thirds power. Hence, a shape-dependent model with a uniform density of turns would have $n = 2/3$ in equation (3).

For the reasons given, a sequence-dependent model seems more likely, and the insensitivity of equation (3) to a choice of n implies that sequence-dependent behaviour need not be at the expense of the protein's globularity. Another way of seeing this

is to consider the surface-to-volume ratio for spheroids of interest, as shown in Fig. 2. Within the size range of proteins, this ratio is nearly constant, and approximately the same for either a sphere or prolate and oblate spheroids with axial ratios of 2:1. That is, the equation

$$A/V = c \quad (4)$$

where A is the surface area, V the volume, and c a constant, is a good approximation over the domain of interest. Since the number of residues in the protein is certainly proportional to the molecular weight which is, in turn, proportional to the volume, a sequence-dependent model will vary linearly with the volume of the protein

$$\text{Turns} \propto V \quad (5)$$

while a shape-dependent model will vary linearly with the two-thirds power of the volume or the surface area

$$\text{Turns} \propto A \quad (6)$$

From equation (4), however, $A = cV$, so the shape-dependent model is also seen to vary linearly with the volume. Thus, within the size range of interest, even a shape-dependent model behaves, to a good approximation, as a linear function of the number of residues.

If geometric criteria alone determine the turns, it is unlikely that any protein of globular shape would deviate very much from the shape-dependent model; while if the local sequence determines turns, a protein of usual composition would be expected to exhibit deviations. Myoglobin is a case in point. Equation (1) predicts 21 turns for the 153 residues of myoglobin, but the algorithm for turns finds only 13. A sequence analysis of myoglobin confirms the unusual composition of this protein which gives rise to unusually long helical structural segments with few turns.

The apparent sequence-dependence in our findings was inferred from counting structural sites in the folded protein. The fact that locally determined, sequence-dependent sites are still discernible after folding can be interpreted to mean that the protein is partitioned into its structural segments and turns by local sequences of amino acids, and that these moieties tend

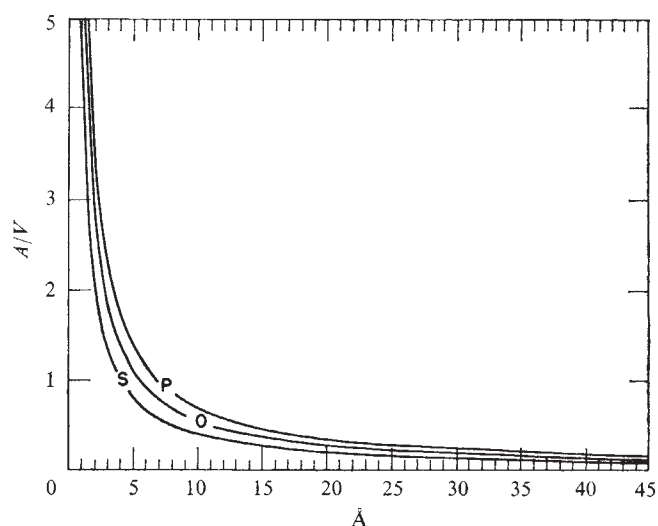


Fig. 2 A plot of the surface-to-volume ratios for a prolate ellipsoid with axial ratio 2:1 (P), an oblate ellipsoid with axial ratio 2:1 (O), and a sphere (S). The abscissa is the radius of the sphere and the length of the major axis of the other spheroids. Assuming a partial specific volume of $0.75 \text{ cm}^3 \text{ g}^{-1}$ and a perfectly spherical shape, rubredoxin would have a radius of 12 Å and LDH would have a radius of 22 Å.

to persist. This interpretation is compatible with our model of protein folding⁶, called the LINC and hinges model. In this model, linearly short and medium range interactions dominate early folding, causing the disordered chain to assemble first into local independently nucleated continuous segments (LINC). LINC are structurally persistent, separable, modular entities that are precursors to their counterparts in the folded protein, and each LINC has its own characteristic equilibrium between random coil and the conformed state. The hinges are viewed as conformationally permissive loci where the backbone changes its overall direction; it is presumed that these become chain turns.

In our folding model, a protein is comprised entirely of LINC and interspersed hinges. These nascent structural elements then coalesce as a result of orientation and diffusion processes to provide a cooperative mutual stabilisation. The cooperative folding of LINC is responsible for the emergence of larger structural domains, and, ultimately, the native structure of the protein. Not until the LINC have coalesced into larger structures does the protein take on its characteristic interior and exterior or its overall globular character. Similar folding models have been proposed by other groups^{7-9,14}.

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GEORGE D. ROSE
DONALD B. WETLAUFER

Department of Chemistry,
University of Delaware,
Newark, Delaware 19711

Received 2 May; accepted 5 July 1977.

- ¹ Kuntz, I. D. *J. Am. chem. Soc.* **94**, 4009-4012 (1972).
- ² Lewis, P. N., Momany, F. A. & Scheraga, H. A. *Proc. natn. Acad. Sci. U.S.A.* **68**, 2293-2297 (1971).
- ³ Rose, G. D. & Seltzer, J. P. *J. molec. Biol.* **113**, 153-169 (1977).
- ⁴ Chothia, C. *J. molec. Biol.* **105**, 1-14 (1976).
- ⁵ Chothia, C. *Nature* **254**, 304-308 (1975).
- ⁶ Rose, G. D., Winters, R. H. & Wetlauffer, D. B. *FEBS Lett.* **63**, 10-16 (1976).
- ⁷ Karplus, M. & Weaver, D. L. *Nature* **260**, 404-406 (1976).
- ⁸ Finkelstein, A. V. & Pitsyn, O. B. *J. molec. Biol.* **103**, 15-24 (1976).
- ⁹ Honig, B., Ray, A. & Levinthal, C. *Proc. natn. Acad. Sci., U.S.A.* **73**, 1974-1978 (1976).
- ¹⁰ Holmquist, R. & Moise, H. *J. molec. Evol.* **6**, 1-14 (1975).
- ¹¹ Schulz, G. E. *et al. Nature* **250**, 140-142 (1974).
- ¹² Janin, J. *J. molec. Biol.* **105**, 13-14 (1976).
- ¹³ Teller, D. *Nature* **260**, 729-731 (1976).
- ¹⁴ Pain, R. *Biochem J.* **155**, 325-330 (1976).

Table 1 No. of turns measured and predicted for 21 proteins

Protein	No. of amino acids	No. of turns (measured)	No. of turns (predicted)
1 Carbonic anhydrase	258	34	34
2 Carboxypeptidase	307	39	41
3 Chymotrypsin	245	36	33
4 Concanavalin A	237	34	32
5 Cytochrome b5	87	10	13
6 Cytochrome C	103	14	15
7 Flavodoxin	138	15	19
8 Glyceraldehyde-3-phosphate dehydrogenase	334	45	44
9 Hexokinase	450	55	58
10 High potential iron protein	85	16	13
11 Lactate dehydrogenase	331	44	44
12 Lysozyme	129	19	18
13 Myogen	108	14	16
14 Papain	212	33	29
15 Phosphoglycerate mutase	218	27	29
16 Ribonuclease S	124	22	18
17 Rubredoxin	53	7	9
18 Serine protease (fragment)	225	31	30
19 Superoxide dismutase	151	21	21
20 Subtilisin	275	37	37
21 Trypsin inhibitor	58	9	10

Measured turns are found with the algorithm of Rose and Seltzer³; these are the data points in Fig. 1. Predicted turns are calculated from equation (1) which gives the best-fit line shown in Fig. 1. The difference between measured and predicted values is the residual for each protein data point.

reviews

Wealth of data on pulsars

S. Jocelyn Bell Burnell

Pulsars. By F. G. Smith. Pp. 239. (Cambridge University: Cambridge, London, New York and Melbourne, 1977.) £9.50.

THE tenth anniversary of the discovery of pulsars occurs later this year and at last the spate of papers seems to be slackening. It is a good time to produce a book on pulsars and we must be grateful to Professor Graham Smith for taking the trouble to do so. This book aims to be a summary and a review of the subject and is at a level suitable for research students in the field, or more senior scientists from other branches of astronomy who wish to have a reference book on the subject.

Its emphasis is mainly on the wealth of observational data that now exists on pulsars, with enough of the theoretical work included to give an idea of how well it all fits together. (It doesn't.) It describes phenomena rather than the theories which are current. There are some topics which the stranger to the subject might not expect to find in a book with this title—for example, several chapters on the interstellar medium, and some X-ray astronomy too. But they are rightly included. I have seen the contents page of another book on pulsars to be published later this year in the USA and it is very striking how similar both these books will be in the topics they choose to consider. Not only is it felt elsewhere too that the time is right to write this sort of book, but the scope of a book on pulsars is clearly determined by the state of the subject today.

The state of the subject is a mass, perhaps an embarrassment, of diverse observational results. The phenomena observed in pulsars are now well authenticated, although it is still hard to see whether differences are differences of degree or differences of kind. Our understanding as to why pulsars are as they are is much more restricted, and it could be some years hence before theoreticians solve some of the horrible problems presented by pulsars. Meanwhile, observers are anxious to find the critical experiments that should be made.

Although the pace has slackened, there have been some significant

developments over the past few years which the author deals with in a variety of ways. For example, the recent conclusion that there is an embarrassingly large number of pulsars to have been formed in supernova explosions gets mentioned a few pages from the end, but throughout most of the book the impression is given that this is undoubtedly the way neutron stars are formed. (In fact one general criticism is that sometimes Professor Smith makes what may be tentative conclusions sound too established.)

The sections on X-ray observations of neutron stars have suffered most. The whole subject is changing very rapidly; even while writing the book Professor Smith admits he had trouble keeping up with it! A couple of years later these sections look very sorry. It must be difficult to judge how and where to draw the line when writing a book containing such material, but one option is not to attempt to include it in any detail. A similar restraint has been necessary until recently on the

whole subject of pulsars.

One would like to recommend this book to a new research student but, although I warmly commend the radio astronomy (the greater part of the book), I would feel obliged to warn him that the X-ray and γ -ray sections are not to be relied upon as accurate accounts of the subjects. There are a number of misprints in the book as a whole which would drive a research student to exasperation, but sorting them out could be quite an educational process in itself. The index on occasion drove me to exasperation. (No Poincaré sphere, Professor Smith!)

The book is very readable with an informal style; it is a welcome compendium of the work that has been done by radio astronomers on and with pulsars. Many of us will find it a useful book for ourselves or others to refer to. We have had to wait a long time for it. □

S. Jocelyn Bell Burnell is a research fellow at the Mullard Space Science Laboratory, University College, London, UK.

Decade of the mouse

Development in Mammals. Vol. 1. Edited by Martin H. Johnson. Pp. 386. (North-Holland: Amsterdam, New York and Oxford, 1977.) \$34.50.

Now that the genetic code has been not only cracked but put to work, the last major uncharted territories of biology are usually reckoned to be the brain and development. The earlier years of the century saw a series of classical experiments on the embryology of invertebrates—for example, sea-urchins and lower vertebrates, in particular amphibians. The past ten years have been the 'Decade of the Mouse'. A wide sweep of technical advances—biochemical, biophysical, microsurgical, and above all advances in the techniques of *in vitro* culture—have rendered the mammalian embryo accessible for the first time to sophisticated analysis. The rapid strides that have been and are being made in our understanding of mammalian development, at the level of mechanisms as well as description, render the field immensely exciting to those of us working in it.

It is therefore timely that a new review series, *Development in Mammals*, has been launched. The editor is Martin Johnson, well known for his personal contribution to our understanding of the immunology of early mammalian embryos; the first two volumes "will concentrate on consideration of peri-implantation embryogenesis and the maternal influences on the embryo".

Volume 1 contains an interesting mix of developmental biology and reproductive physiology, two disciplines that in the past, in spite of their obvious overlap, have had inadequate contact. Reproductive physiologists have concentrated on the female and the male, often forgetting that reproduction implies an embryo too; developmental biologists, because of their traditional pre-occupation with non-mammalian material, sometimes find it hard to come to grips with the endocrinological, physiological and anatomical complexities of the maternal-embryonic relationship in mammals. This volume should go far to bridge the gap.

The biochemical and biophysical as-

pects of pre-implantation development are comprehensively covered by T. Ducibella (surface changes and cell-cell interactions in trophoctoderm), R. M. Borland (transport processes in the mammalian blastocyst, the trophoctoderm as an epithelium), G. A. Schultz and E. B. Tucker (protein synthesis and gene expression) and C. M. Warner (RNA synthesis and RNA polymerase activity). On the reproductive side, J. E. O'Grady and S. C. Bell provide an exhaustive review of uterine aspects of implantation; M. A. H. Surani describes protein changes in uterine fluids at the time of implantation; R. J. Aitken deals with delay of implantation, and the signals that may switch embryo and uterus on and off; M. Beato discusses the synthesis of one particular uterine protein, uteroglobin. M. H. Kaufman reviews his work on the effects of anaesthetics on gametogenesis and post-implantation development, in the context of clinical studies.

It is sad that the only hint of the intellectual excitement of the field, and of the beauty and amazing ingenuity of the mammalian embryo, comes in the Editor's introduction. The articles themselves tend to get bogged down in experimental detail, often contradictory. One consequence of selecting authors who are themselves actively working in a rapidly moving area is that one gets up-to-the-minute information, much of it still unpublished; a less desirable consequence is that the viewpoint is inevitably one-sided. Some of the articles are definitive

reviews of a topic (Aitken; O'Grady and Bell) but most are of the nature of progress reports. Such a collection requires stringent editing. Able research workers are not necessarily good writers; Dr Johnson fields an impressive team but allows them too much freedom.

Many linguistic errors and misprints mar the pages: I spotted 54 in one 80-page article, without picking any editorial nits over hyphens or punctuation. The heterogeneity of misprint-density suggests that proof-reading was left to individual authors. There are also infelicities such as "Neither results strongly support . . ."; all 'less than' signs are omitted from probability levels in one chapter; and why choose a typeface that distinguishes 1 from l if you then abbreviate chlorine as Cl throughout? The typed text is photographically reduced to a size that I (and others to whom I showed the book) found tiring to read.

These are minor criticisms. I wholeheartedly welcome the appearance of the new series, and congratulate publishers and Editor on their enterprise. I urge anyone with an interest in developmental biology or reproductive physiology to buy this volume, and to get their libraries to buy it too. It is worth the money for the comprehensive reference lists as well as for the articles themselves.

Anne McLaren

Anne McLaren is Director of the MRC Mammalian Development Unit at University College, London, UK.

Antinucleon physics

Antinucleon-Nucleon Interactions. Edited by G. Eskpong and S. Nilsson. Pp. xvii+600. (Pergamon: Oxford and New York, 1977.) £22.

THE ANTIPROTON was discovered in 1955 and the antineutron a year later, so antinucleons have only recently come of age. The interaction of these particles with nucleons is an active research field, and in the past few years has been the subject of a number of specialised symposia of which the latest was held in Stockholm in July 1976. These published proceedings of this meeting contain 8 invited review papers and about 50 research contributions. The discussion following each talk has not been recorded.

The existence of antiparticles suggests the intriguing possibility of bulk antimatter in the Universe. A cosmology symmetric between matter and antimatter has been championed for a number of years by H. Alfvén (Stockholm) who reviewed this topic. There is no evidence

for galaxies or other distant regions of antimatter, but neither can their existence be ruled out from direct experimental measurements.

All other talks came down to Earth and covered aspects of antinucleon behaviour in the laboratory. The construction of separated antiproton beams of high intensity, in particular at the CERN and Brookhaven proton synchrotrons, has greatly enhanced the scope of low and medium energy experiments in the last few years. Antiprotons transported at a few hundred MeV/c can be brought to rest in targets and used to study the $\bar{N}N$ system at very low energies. This topic was reviewed by T.E.O. Ericson (CERN). Theoretical models predict the existence of $\bar{N}N$ states bound by the strong nuclear interaction, but these have not been established definitely by experiment. Antiprotons can, however, certainly be bound to nuclei by the Coloumb force to form antiprotonic atoms. X-ray transitions between levels have been studied and a number of interesting results obtained including a measurement of the antiproton magnetic moment.

Formation experiments are a powerful

technique for studying hadron resonances. At the appropriate centre of mass energy, the colliding particles coalesce briefly to create a single particle, which then decays. To study possible meson formation, a conventional nucleon target requires a beam of antibaryons, in practice antiprotons. A clear review by E. Eisenhandler (Queen Mary College, London) outlined the considerable recent progress in this field. A few years ago suggestive enhancements were seen at various mass values in the antiproton-nucleon total cross sections. More recently these bumps were seen also in the $\bar{p}p$ elastic channel. The investigation of other channels, in particular two-meson final states provides much additional information. Angular distributions for $\bar{p}p \rightarrow \pi^0\pi^0$; $\eta^0\eta^0$; $\pi^0\eta^0$ were presented by a Bari-Brown-MIT collaboration, and results for polarisation in $\bar{p}p \rightarrow \pi^+\pi^-$; K^+K^- by a QMC-Daresbury-Rutherford collaboration.

When the Fermilab accelerator started operation, antiprotons in the 100 GeV/c region were produced quite copiously. At these momenta, however, particle separation using electrostatic or even RF fields becomes prohibitively difficult. An ingenious scheme has been used where antiprotons arising from antilambda decay in a short neutral beam were then transmitted to the experiment. At 100 GeV/c, annihilations are reduced to perhaps only a tenth of all $\bar{N}N$ interactions. These annihilations were reviewed by J. G. Rushbrooke (Cambridge). Experiments are still exploratory, measuring multiplicities, topological cross sections and inclusive production spectra. Annihilations at medium energies, and related processes, were covered by J. R. Fry (Liverpool). High energy non-annihilation interactions, where baryon and antibaryon are still present in the final state, were reviewed by S. Nilsson (Stockholm).

Two invited theoretical reviews were given. H. Miettinen (SLAC) discussed progress in antiproton physics mostly within the framework of quarks and duality diagrams. Both he, and also G. F. Chew (Saclay and Berkeley) in a more speculative review, considered the possibility of 'baryonium' states which do not have the conventional quark-antiquark structure of mesons.

Some plans for future facilities were outlined in contributed papers, but unfortunately the symposium came a little too early to include recent ideas on antiproton beam cooling and the possibility of storing antiprotons and colliding them with protons, thus giving rise to collisions at superhigh energies.

The editors and publisher have produced an attractive volume which should be of considerable interest to specialists in the field. **P. I. P. Kalmus**

P. I. P. Kalmus is Reader in Experimental Physics at Queen Mary College, University of London, UK.

Platelet research

Platelets in Biology and Pathology. Edited by J. L. Gordon. Pp. 388. (North-Holland: Amsterdam and New York, 1977.) \$53.95; Dfl. 132.

THIS book is the first volume in a new series *Research Monographs in Cell and Tissue Physiology*, with J. T. Dingle as general Editor. This volume, written by recognised cognoscenti in the various areas of platelet research, may be considered as a collection of progress reports over the past decade's research, with emphasis on current work. J. L. Gordon is responsible for the excellent selective editing.

Few scientists, if any, working with platelets, can keep abreast of the progress in this very active and rapidly developing field. B. Maupins annotated bibliography on blood platelets, for example, contains about 1,300 references to work published in 1972. The authors of the present monograph have very effectively filtered the plethora of material and offer the reader a comprehensive survey of the present state of knowledge; they provide roughly 1,600 selected and useful

references.

The book is divided into two parts. Part one comprises an outline of fundamental platelet reactions, part two deals with platelet constituents, cellular control mechanisms and their biological significance. The first chapter in part one, "Blood platelets as multi-functional cells" (J. L. Gordon and A. J. Milner) is a well written appetiser, very fitting to the contents of the book. I am convinced that scientists to whom platelet research may seem a somewhat restricted subject, will acquire by reading these pages an informed interest in the numerous biological phenomena in which platelets are involved. The remainder of part one deals with platelet adhesion and aggregation (Baumgartner and Muggli) and the platelet release reaction (MacIntyre).

The book's second part brings together a great deal of valuable information on the platelet plasma membrane (Jamieson and Smith), lipids (Deykin), filaments and microtubules (Crawford), various platelet receptors (Mills and Macfarlane), biogenic amines (Drummond), platelets and thrombin (Majerus *et al.*), connective tissue and platelets (Jaffe), β -thromboglobulin (Moore and Pepper), platelets in immunological reactions (Brown),

prostaglandins (Smith and Silver), platelet proteinases (Ehrlich and Gordon) and platelet growth-stimulating factor (Ross *et al.*).

In general, the chapters are well balanced; they bring out points of significance and offer a great deal more than a mere catalogue of original sources of information. It is to be hoped that some of the messages of the book will be widely appreciated—namely, that we still know next to nothing about the physiological importance of the many *in vitro* platelet phenomena so eagerly studied at present; that enough information has accumulated on interspecies differences in platelet chemistry and behaviour to make generalisations difficult or impossible; and that animal models for the study of the pathogenesis of human pathological conditions involving platelets are therefore very difficult to establish.

I found this monograph stimulating and I recommend it to anyone wishing to acquire an overall impression of the major developments and current lines of thought in the platelet field.

O. Behnke

O. Behnke is Professor at the Institute of Anatomy, University of Copenhagen, Denmark.

Parasitic protozoa

Parasitic Protozoa. Vol. 1: Taxonomy, Kinetoplastids and Flagellates of Fish. Edited by Julius P. Kreier. Pp. xv+441. (Academic: New York and London, 1977.) \$35; £24.85.

THIS volume is the first of a series of four which are planned to present, in all, 40 chapters written individually by experts in each particular field and covering mainly the parasitic Protozoa but including also a number of organisms which are not, or only doubtfully, Protozoa, such as *Anaplasma*, *Eperythrozoon* and *Pneumocystis*. This is not so incongruous as might appear at first sight, as these organisms resemble the Protozoa in multiplying in their vertebrate host. The scope is, in fact, a welcome change from the more usual association of the Protozoa with only the helminths under the blanket term 'parasitology'.

The present volume contains 10 reviews, rather variable in length from about 25 to about 75 pages. The first two deal with taxonomy—respectively, with the position of the phylum Protozoa in the Animal Kingdom and with the internal classification of the phylum. The remainder deal exclusively, or predominantly with kinetoplastid flagellates and are rather variously defined. There are two defined purely taxonomically—the reviews of

Leishmania and *Trypanosoma cruzi*. *Trypanosoma* is otherwise treated in six reviews defined by pathogenicity in relation to host and to geographical distribution—those species causing diseases in man and other primates, or in cattle in sub-Saharan Africa or elsewhere, and those species generally regarded as 'non-pathogenic'. Finally, there is a treatment of the flagellate parasites of fish in which kinetoplastids figure largely but which also include dinoflagellates and polymastigids.

All the reviews are very readable, comprehensive and accurate treatments and (assuming their scope) are excellent summaries of the present states of knowledge for each field. Within the space provided for each review it is not possible to go into much discussion of matters which are still controversial, and so some statements will inevitably seem dogmatic on wider reading. Nevertheless, the reviews are all excellent lead-ins to their subjects and will be very useful for students and for research workers. The book is well produced, referenced and indexed. Its cost, nearly £25, seems high, even in these times, as it is only rather sparsely illustrated with a few line drawings and photographs.

W. H. R. Lumsden

W. H. R. Lumsden is Professor and Head of the Department of Medical Protozoology at the London School of Hygiene and Tropical Medicine, London, UK.

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Vertebrate evolution

Problems in Vertebrate Evolution. Edited by S. Mahala Andrews, R. S. Miles and A. D. Walker. Pp. xi+411. (Academic: London and New York, 1977.) £18.50; \$36.10.

THIS volume of thirteen papers by vertebrate palaeontologists is a mark of their respect and regard for Professor Stanley Westoll on the occasion of his retirement. The subjects covered are, like his own interests, centred on fishes but extend also into the functional anatomy of tetrapods.

The commonest theme is the problem of comparative anatomy, in the sense of the search for the methods of establishing homology between structures in different groups, and also the application of these methods. Schaeffer develops the interesting and basic hypothesis that the dermal skeleton is always the result of an interaction between the epithelium and the underlying mesenchyme, and that its different manifestations (as enameloid, enamel, dentine or membrane bone) are merely the results of changes in the timing, duration or number of steps in the series of morphogenetic events. In another important paper, Patterson concludes that, contrary to current belief, there is no interchangeability between membrane bones and cartilage bones (though the two may fuse together), and that superficial "dermal" bones must be distinguished from membrane bones, the latter ossifying deep in the mesoderm. Ørving gives a comparative survey of the superficial dermal structures, tooth-like in appearance though not in position or function, that he calls odontodes. Gardiner and Bertram discuss the braincases of two new palaeoniscids and conclude that the ventral cranial fissure represents the gap between the ossifications in the trabecular-polar bar and the basal plate. Thomson discusses the ontogeny of cosmine, and suggests that the porecanal system of Palaeozoic fishes had an electrosensitive function. Finally, Whiting returns to the problem of the identification of the cranial nerves of cephalaspids, and provides considerable evidence that Stensiö's numbering of these should be revised.

The often bewildering interplay between ontogeny, functional anatomy and phylogeny is well shown by Panchen's work on the variety of vertebral structure in early tetrapods, and by Mahala Andrews' paper on the implications of some new features in the axial skeleton of *Latimeria*—since we still know virtually nothing of it as a living creature, its frequent mention

as a "living fossil" in popular articles is rather ironic! On a related topic, Parrington suggests that intercentra were retained in the neck of cynodonts to increase the flexibility of this region. Finally, Walker provides some new ideas on the vexed question of the relationships between the functional morphology of the pelvic girdles of birds and dinosaurs (and, by implication, the relationships of their owners).

There are three papers primarily concerned with systematic work. Miles and Young describe new ptyctodontid placoderms from Australia, and provide arguments for a new classification of placoderms in which the arthrodires (*sensu stricto*) and antiarchs are closely related to one another. Jarvik finds a surprising degree of similarity between the detailed cranial anatomy of *Acanthodes* and that of living sharks, and

suggests that these two groups are far more closely related than had been thought. Finally, Carroll explores the implications of his recent work on the earliest known (Permo-Triassic) lizards, discussing the basic adaptive pattern and early evolution of the group; he also suggests that sphenodontids are more closely related to the lizards than to the rhynchosaurs.

The volume has an attractive format and a good index. None of the papers in it are trivial, most are well-written (though occasionally blemished by imperfect proof-reading), and the volume deserves a place on the bookshelf of anyone working on the comparative anatomy and evolution of fishes.

Barry Cox

Barry Cox is Professor of Zoology at King's College, University of London, UK.

Ultraviolet photoelectron spectroscopy

Principles of Ultraviolet Photoelectron Spectroscopy. By J. Wayne Rabalais. Pp. xv+454. (Wiley Interscience: New York and London, 1977.) \$36; £22.50.

THIS is really a very good book which will be useful to photoelectron spectroscopists for many years to some—no mean achievement in such a rapidly changing research field. The author has not fallen into the trap of undue concentration on spectra of specific molecules or indeed of paying special attention to particular research areas within the ultraviolet photoelectron spectroscopy (PES) field. Rather, there has been a deliberate policy of concentrating on the fundamental principles of the various effects which are encountered in PES and for once the book's title is a true reflection of the content. In my opinion, the author was also correct in concentrating on the vacuum ultraviolet aspects of photoionisation electron spectroscopy, a subject which provides direct information on the electronic structure of molecular ions, rather than diluting the contents in an attempted comprehensive electron spectroscopy text.

The detailed content of the book is significantly different from that found in existing texts, and the degree of overlap is remarkably low. A suitably short introduction is followed by a brief but adequate chapter on experimental methods. Chapter three is a useful one in that it brings together, in a mathematical way, a number of related topics such as photoionisation, Franck-Condon factors, isotope effects, selection rules and configuration inter-

action. The next chapter deals with the theoretical methods used to describe and quantify ionisation from closed-shell molecules. This chapter is adequate, although a colleague thought that a discussion of the more recent molecular orbital methods such as the *ab initio* many body approach, the equations of motion method and the application of time-independent perturbation theory to the problem of calculating ionisation energies could have been included to advantage.

The next three chapters are ones which are not standard content in books on PES and for this reason are especially useful to researchers in the field. Chapter five deals with the special aspects of ionisation from open-shell molecules in a mathematical fashion, and includes a good account of the vector-coupling method for open shells. Chapter six deals with the theory of photoionisation cross-sections in as reasonable a way as is at present possible, since considerable development work is still required in this area. The following chapter deals with the interpretation of photoionisation cross-sections and angular distributions within the limits of our present day understanding.

Chapter eight is a detailed account of spin-orbit coupling in molecular ions and chapter nine is concerned with configurational instability of molecular ions—basically, the Jahn-Teller and Renner effects. On balance, the topics in chapter nine are discussed in rather more detail than one might justify from their importance in experimental PES. The last two chapters are more conventional and are basically concerned with experimental results and their interpretation.

Neville Jonathan

Neville Jonathan is Professor of Chemistry at the University of Southampton, UK.

Recent scientific and technical books

Applied Biology

- BAKHLE, Y. S., and VANE, John R. (edited by). *Metabolic Functions of the Lung*. (Lung Biology in Health and Disease, Vol. 4.) Pp.xvi+353. ISBN-0-8247-6383-1. (New York and Basel: Marcel Dekker, Inc., 1977.) SFr.112.
- BARLTROP, Donald (edited by). *Paediatric Implications for Some Adult Disorders*. (Scientific Proceedings of the 4th Unigate Workshop held at the Royal College of Physicians, St. Andrew's Place, NW1, May 1976.) Pp.vii+152. ISBN-0-9501839-3-8. (London: Fellowship for Postgraduate Medicine, 1977.) £4.
- BAUR, Rolf. *Morphometry of the Placental Exchange Area*. (Advances in Anatomy, Embryology and Cell Biology Vol.53, Part 1.) Pp.65. ISBN-3-540-08159-3. (Berlin and New York: Springer-Verlag, 1977.) DM32; \$14.10.
- BECK, William S. (edited by). *Hematology*, Second edition. Pp.xi+589. ISBN-0-262-52038-9. (Cambridge, Mass. and London: The MIT Press, 1977.) \$9.95.
- BECKER, Frederick F. (edited by). *Cancer: a Comprehensive Treatise*. Vol. 5: Chemotherapy. Pp.xvi+666. ISBN-0-306-35205-2. (New York and London: Plenum Press, 1977.) \$51.
- BERGSMA, Daniel, and LENZ, Widukind (edited by). *Morphogenesis and Malformation of the Limb*. (The Second International Conference on Morphogenesis and Malformation, held at Tübingen, Federal Republic of Germany, Sponsored by The National Foundation—March of Dimes.) (Birth Defects: Original Article Series, Vol. 13, No.1.) Pp.xi+364. ISBN-0-8451-1008-X. (New York: Alan R. Liss, Inc., 1977.) \$35.
- BIRCH, Gordon G., SPENCER, Michael, and CAMERON, Allan G. *Food Science*. Second edition. (Pergamon International Library of Science, Technology, Engineering and Social Studies.) Pp.viii+191. ISBN-0-08-021347-2. (Oxford and New York: Pergamon Press, 1977.) Hard cover £6.95; Flexi covers £3.95.
- BLANDAU, Richard J., BERGSMA, Daniel (edited by). *Morphogenesis and Malformation of the Genital System*. (The Third International Conference on Morphogenesis and Malformation, held at Lake Wilderness, Washington, July 1976.) Sponsored by The National Foundation—March of Dimes. (Birth Defects: Original Article Series, Vol. 13, No.2.) Pp.xi+61. ISBN-0-8451-1009-8. (New York: Alan R. Liss, Inc., 1977.) \$35.
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- CHRISPEELS, Maarten J., and SADAVA, David. *Plants, Food, and People*. (A Series of Books in Biology.) Pp.278. ISBN-0-7167-0378-5. (San Francisco: W. H. Freeman and Company, 1977.) Cloth \$12; Paper \$6.50.
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- SMITH, J. E., CLAPHAM, A. R., and RATCLIFFE, D. A. (organized by). *Scientific Aspects of Nature Conservation in Great Britain*. (A Royal Society Discussion held in 10 June 1976.) Pp.ix+103. ISBN-0-85403-089-1. (London: The Royal Society, 1977.) UK £3.50; Overseas £3.65.
- SILVER, Melvin J., SMITH, J. Bryan, and KOCIS, James J. (edited by). *Prostaglandins in Hematology*. (Proceedings of the International Symposium, Philadelphia, March 4-5, 1976.) (Monographs of the Physiological Society of Philadelphia, Vol. 3.) Pp.416. ISBN-0-89335-014-1. (New York: S.P. Books Division of Spectrum Publications, Inc., 1977.) \$30.
- SMITH, Louis D. S. *Botulism: The Organism, Its Toxins, The Disease*. (A Monograph in The Bannerstone Division of American Lectures in Clinical Microbiology.) Pp.xiii+236. ISBN-0-83043-431-1. (Springfield, Illinois: Charles C. Thomas, Publisher, 1977.) \$18.75.
- SPENCER, H. *Pathology of the Lung (Excluding Pulmonary Tuberculosis)*. Third edition. Vol. 1: Pp.xiii+1-542. Vol. 2: xi+543-1099. ISBN-0-08-021021-X. (Oxford: Pergamon Press, 1977.) £47.50.
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- TURUSOV, V. A. (editor-in-chief). *Pathology of Tumours in Laboratory Animals*. Vol. 1: Tumours of the Rat, Part 2. Pp.viii+318; ISBN-92-832-1106-5. (Lyon: International Agency for Research on Cancer, 1976.) Distributed by WHO, Geneva. S.Fr.90; \$35.
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- VIROLOGY IN AGRICULTURE. (Beltsville Symposia in Agricultural Research, 1). (Invited papers presented at a Symposium held May 10-12, 1976, at The Beltsville Agricultural Research Center, Beltsville, Maryland.) Pp.xiv+293. ISBN-0-85626-137-8. (Tunbridge Wells, Kent: Abacus Press; Montclair, NJ: Allanheld, Osmun and Co. Publishers, Inc., 1977.) £15.80.
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- WILSON, James G., and FRASER, F. Clarke (edited by). *Handbook of Teratology*, Vol. 2: Mechanisms and Pathogenesis. Pp.xi+491. ISBN-0-306-36242-2 (v.2.) (New York and London: Plenum Press, 1977.) \$46.80.
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KOVEL, Joel. *A Complete Guide to Therapy: From Psychoanalysis to Behaviour Modification*. Pp.xviii+284. ISBN-0-85527-929-X. (Hassocks, Near Brighton, Sussex: The Harvester Press, Ltd., 1977.) £5.95.

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General

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obituary

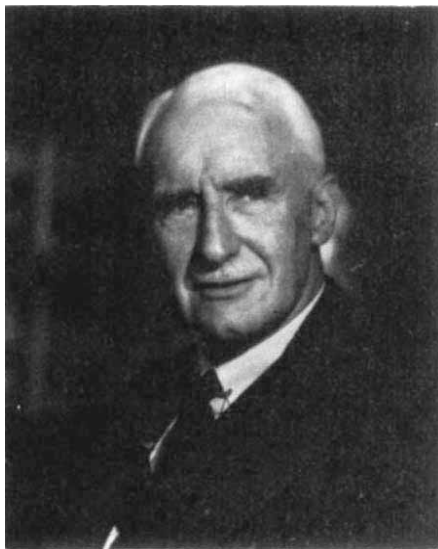
A. V. Hill

A. V. HILL died at his home in Cambridge on 3 June, in his 91st year. A.V., as he was known to his colleagues all over the world, was one of the great scientific leaders of his generation. He will be remembered not only for his pioneering work on the energy exchanges in active nerve and muscle, but for bringing physico-chemical ideas and precision measurements to bear on biological problems, and above all for his personal example and influence in inspiring and encouraging many of his younger colleagues, and in helping those who had been driven from their laboratories by political or racial persecution.

He was born at Bristol on 26 September 1886, went to Blundell's School at Tiverton, and in later years maintained a close connection with the West Country. He used to spend many summer months in Devonshire, staying with his family at 'Three Corners', his little house in Ivybridge, and working from time to time at the nearby Marine Biological Laboratory at Plymouth. In 1913, A.V. married Margaret Keynes, sister of John Maynard and Geoffrey Keynes, starting a partnership which lasted for nearly 60 years, and a pleasant family home whose hospitality is remembered by colleagues and friends from many countries.

At Blundell's School, he was profoundly influenced by J. Thornton, an outstanding mathematics teacher who also taught his pupils the need for clarity in thought and precision in the use of words. In 1905, A.V. went to Trinity College, Cambridge as a Scholar, taking mathematics and finishing as 3rd Wrangler in 1907. Under the tutelage of W. M. Fletcher (later the first Secretary of the Medical Research Council), he decided to turn to physiology and in 1909 took a first in part II of the Natural Sciences Tripos. The year after, he was made a Fellow of Trinity College.

In the Physiological Laboratory at Cambridge, A.V. found himself in the company of outstanding people, among them Joseph Barcroft, Keith Lucas, J. N. Langley, Walter Fletcher and F. Gowland Hopkins. He started by publishing theoretical papers on nerve excitation and on the kinetics of the oxygen-haemoglobin reaction. The latter is still referred to on occasions,



when authors use the so-called 'Hill-coefficient' as a characteristic index in aggregate or cooperative molecular interactions. But before long, A.V. embarked on his own experimental research which he pursued, with interruptions caused by two world wars, for the next 55 years.

He followed Langley's advice to investigate 'the efficiency of the cut-out frog's muscle as a thermodynamic machine.' This became an unending story, in which he was helped by his assistants A. C. Downing and J. L. Parkinson, developing instruments of increasing sensitivity, greater time resolution and better stability, a story of continuously searching for errors in technique and interpretation, of replacing obsolete results and ideas with better experiments and fresh hypotheses; it was a process of temporary setbacks and continuously improved experimentation that gave A.V. the greatest pleasure and provided him with the kind of entertainment he enjoyed throughout his life. His early measurements of heat production in active muscle, analysing and dividing it into an initial anaerobic, and a subsequent, oxygen-requiring recovery phase, correlating them with what was known about lactic acid production and oxidative restoration processes, is now part of classical history, but at the time it was a most exciting technical and experimental advance and represented one of the outstanding contributions to our knowledge. Moreover, A.V. was quick in extending the work and applying it to the study of muscular exercise

and the associated oxygen requirements in man.

Hill's preoccupation with precise and exceedingly sensitive myothermic measurements had some important 'spin-off.' It enabled him to detect the heat production during the conduction of nerve impulses, a feat which many distinguished scientists, among them the great Helmholtz, had attempted, but failed to bring off. In addition, A.V. showed that his thermopiles could be applied to practical problems, in measuring osmotic pressure of very small volumes of body fluids, and in determining arterial elasticity and pulse wave velocity. Although he once publicly admitted that he did his work 'not because it is useful, but because it's amusing', he was nevertheless accustomed to looking at living machinery with the eyes of a practical engineer. This, together with his personal interest in athletics, prompted him to study muscular movement not only in isolated frog muscles, but in man, and to work out the relations between physical dimensions of different animals and the limits to the speed and power of their muscular performance.

Hill's early work in the Cambridge laboratory came to a halt in 1914; he joined the Cambridgeshire Regiment, served as Captain and Brevet Major and later became Director of the Anti-Aircraft Experimental Section, another useful outlet for his engineering interests. He recommenced his muscle experiments in Cambridge at the end of the war, but in 1920 went to the Physiology Chair in Manchester. Three years later, he moved to University College London, first as Jodrell Professor of Physiology, then as Foulerton Professor of the Royal Society. He stayed at UCL, working in the laboratory for many years after his official retirement, until 1967 when he returned to Cambridge, leaving behind a Biophysics Department which he had founded in 1951.

A. V. Hill gave public service of many different kinds. During the uneasy years before 1939, he joined the Tizard Committee which initiated radar defence, and he also accepted the task of compiling a Central Register of Scientific and Technical Personnel in anticipation of wartime needs. He did this during a period when he was fully engaged in his laboratory, working

single-handed on new experiments which resulted in the publication of one of his most important papers, on the energy liberation and the heat produced during shortening of active muscle. At the same time, he served as Biological Secretary of the Royal Society and on numerous scientific committees, and took a leading part in helping refugee scientists.

During the 1939/45 war he was Member of Parliament for Cambridge University, and he served as Scientific Adviser to the Government of India in 1943-44. To name only some of his other extramural activities, he served the Physiological Society, on its Committee, and as an Officer and Editor of its Journal, for 25 years, was Foreign Secretary of the Royal Society, Secretary General of the International Council of Scientific Unions, President of the British Association in 1952 when he gave his memorable address on 'The Ethical Dilemma of Science', and Chairman of the Society for the Protection of Science and Learning. A.V. gave a powerful stimulus to the study of marine biology in this country. He was President of the Marine Biological Association, and he constantly encouraged his younger colleagues to work at Plymouth and take advantage, not only of its delightful surroundings, but of the unique opportunities for experiments provided by the marine fauna. This initiative, together with his fascinating Liversidge Lecture on 'Chemical Wave Transmission in Nerve' helped to attract a number of distinguished biologists to the Plymouth Laboratory which later on led to very striking developments in the neuro-physiological field.

A.V. was honoured by academic institutions all over the world; he was made a Companion of Honour in 1946 and received high honours from the Governments of France and the United States. At the age of 31 he was elected to the Royal Society who later awarded him the Royal and Copley Medals, and he received the 1922 Nobel Prize for Physiology together with Otto Meyerhof.

He is remembered and held in affection by all his pupils, not only as a great scientist who imparted to them the joy and spirit of adventure in doing experiments, but as a man who gave an example of uncompromisingly straight dealing, in the laboratory as well as outside. The pursuit of truth was to him not simply a scientific exercise, confined to one's work in the laboratory and presentation of results; it also governed his attitude in all his actions and in his relations to other persons. He was unrelentingly critical and never ceased to probe for flaws in his own deductions or experimental

approach. 'To be uncritical', he said, 'particularly of oneself and one's ideas and motives, is the first long step towards dishonesty.' Yet, he never permitted his intellectual austerity to damp his enthusiasm, and I do not believe he ever allowed the slightest doubt to arise in his mind about the importance of the work he was engaged in. This buoyancy transmitted itself to those who were associated with him and helped to carry them over periods of failure and possible frustration. A.V. liked to get a good laugh out of situations which others might have regarded as perhaps slightly embarrassing; he detected and managed to focus on the humorous side of people's failings including his own. He retained, until nearly the end, a delightful, boyish sense of humour which greatly appealed to most of his friends, though at times it may have puzzled the more serious-minded ones among them.

I shall always remember a particular occasion in 1934. I was then a medical student in Leipzig, about to take my finals and anxiously making plans to escape from a hostile environment. I came across a correspondence in *Nature* between A. V. Hill and Johannes Stark, a famous physicist who had become the scientific 'Gauleiter' in Nazi Germany. After the 1914-18 war, A.V. had insisted (against some initial resistance) that colleagues from Germany should be invited straight away to attend International Congresses and be made to feel welcome in the scientific community. But in 1933 he was also the first to speak out vigorously against the Hitler regime and the dismissal and persecution of Jewish scientists. He was promptly taken to task by Professor Stark who stated in two letters to *Nature* that there was no factual basis for A.V.'s critical remarks, that the Nazi Government was obliged in self-protection to restrict the influence of certain persons and was simply engaged in lawful activities like any other respectable Government. The correspondence ended with a short note from A.V. in which he said that monetary contributions had been received in response to his appeal for assistance to colleagues who had been driven out of Germany. He added that he was not quite certain whether the donations were the result of his own eloquence, or rather of the explanations given by Professor Stark to whom, he felt some thanks were due. The *Nature* correspondence gave me my first impression, from a distance, of A.V.'s personality, and I found it so attractive that I made every effort to go and work with him as soon as I could.

There will be hundreds of scientists from different continents who recall with gratitude the welcome and hos-

pitality they received, not only in the laboratory in Gower Street, but from A.V. and Margaret at their home in Highgate and at 'Three Corners' in Devonshire. Last year, on his 90th birthday, well over a hundred of his friends sent him a large volume containing greetings and reminiscences, and A.V. very much enjoyed browsing through them during the remaining months of his life. There can be few scientists and teachers who have won as much affection as A.V., and I believe that his personal influence among his scientific descendants in all parts of the world is a contribution to international scientific relations, not on a large scale, but of a kind perhaps more valuable than anything that official organisations could provide.

B. Katz

Roman Kozlowski

PROFESSOR ROMAN KOZLOWSKI, who died on 2 May, 1977, at the age of 88, was perhaps the most internationally honoured doyen of invertebrate palaeozoology. He was responsible for establishing a school of palaeontological study at Warsaw of exceptional breadth and excellence. His major contributions were on the Brachiopoda and Graptolithina, but he made some contributions on most other invertebrate fossil groups.

He was born on 2 February, 1889, at Wloclawek, north-west of Warsaw, Poland, and in 1907 he attended the University of Freiburg, and continued his geological and biological studies at the Sorbonne where he graduated in 1910. Between 1913 and 1921 Kozlowski was Professor of Geology and Mineralogy and later Director of the School of Mines at Oruro, Bolivia. It was during this period that his early work on the Carboniferous Brachiopoda and Devonian faunas of Bolivia was mostly done. The latter field he developed for a doctoral thesis under Professor Marcellin Boule (whom he affectionately spoke of as 'mon Maitre') which he received in 1923: the monograph, published in the *Annales de Paléontologie*, set a new standard for palaeontological illustration by collotype.

In 1924 he returned to Poland to teach at the University of Warsaw, becoming a full Professor of Geology and Palaeontology in 1934 and Chairman of the Palaeontology Department. Then really began the progressive growth to excellence of his department. His first major subsequent work followed visits in 1925 and 1926 to the Dnieper River

sections in Podolia. The resulting monograph *Les brachiopodes gotlandien de la Podolie polonaise*, has been described by Shirley as 'a source of inspiration for a generation'; but this referred to the impeccable standard set by his brachiopod descriptions, especially of their internal structures. It was seminal in another way, since it gave details of a marine sequence passing from the Silurian into the Devonian. The area has recently been critical for the international definition of the Silurian/Devonian boundary.

In 1931 he noticed that graptolites were preserved in translucent chalcidony in Tremadocian rocks of the Holy Cross Mountains, and he discovered how easily these could be extracted by dissolution in hydrofluoric acid. This led eventually to the discovery of three new groups of graptoloid, and the discovery of much new detail on the ontogeny of the dendroid graptolites, and also the development of immaculate serial sectioning techniques. It also led to the extension of the techniques to other periods and a corresponding range of contributions on other groups.

The story of the survival of Kozłowski's manuscript for his monograph on Polish Tremadocian graptoloids is a most harrowing tale. His work was completed in 1939 and the bombing of Warsaw imperilled his laboratory, so he moved the typescript, illustrations and most of the specimens to a basement below the Seismological Conservatoire: his institute was destroyed. The Conservatoire was also destroyed, but a month later he was able to enter the basement, but found it sacked, and his material missing. Several months later he found part of his original manuscript in the university ruins, and shortly after, his friend Dr Rozkowski, who was later assassinated, discovered the remainder buried under snow in a courtyard.

At the Warsaw uprising of August 1944 Kozłowski was expelled, but first he hid his manuscript in the central heating system of a private house and, although the house was ruined, he was able to rescue the manuscript in 1945. The originals for his plates were lost, but since he had taken the precaution of sending negatives of these to Paris in 1939, he was able to publish the monograph in 1949. Immediately, this established a new reputation for him in the field of the graptoloids. Particularly important was the detailed case he argued for assigning this important fossil group to the Pterobranchia of the Hemichordata.

After the Second World War he returned to teaching and built up his research school again and until 1960, when he retired, he was Chairman of

the Palaeontological Institute of the Polish Academy of Sciences in Warsaw. When the writer visited him in 1962 he was enthusiastically engaged in preparations for the Polish Mongolian expeditions which have led to so many valuable studies under the direction of his former student Professor Kielan-Jaworowska.

The Geological Society of London awarded him the Wollaston Medal in 1961; he was an honorary member of many Polish and international scientific societies, an honorary graduand of the universities of Krakow, Paris and Modena, a member of the Academies of Science of Poland, Czechoslovakia, France and Colombia; he was a Polish Scientific Prize-winner of the first degree.

The most important other monuments to the work of Roman Kozłowski are the two periodicals which he founded, namely, *Palaeontologica Polonica* of which he edited thirty-four volumes between 1929 and 1976, and *Acta Palaeontologica Polonica* which he edited from 1956 to 1974. These record the incredible breadth of research work done largely by himself and his students and research associates, and give an indication of his infectious enthusiasm for all branches of palaeobiology.

Michael House

George Cotzias

ON June 13, 1977, just 3 days before his 59th birthday George Cotzias died of lung cancer. With his untimely passing the world lost a prominent neurobiological investigator who had made a number of fundamental contributions to the field.

Born in Athens, Greece, the son of a prominent political figure, he fled to the United States in 1941 when his country was occupied during World War II. His medical education which had begun at the University of Athens was continued at Harvard where in 1943 he was awarded the MD degree. Dr Cotzias then served as a house physician on the neurological and medical services of the Massachusetts General Hospital following which he began his career in research at the Rockefeller Institute in New York City. His initial interest centred on renal function but he soon became intrigued with the biological role of trace metals in tissue metabolism particularly in regard to the nervous system.

I remember well my first contact

with Dr Cotzias in the 1950s. He was working on the mechanisms by which enzymes involved in oxidative phosphorylation were activated by manganese and how this might alter central nervous system function. I was organising a symposium on parkinsonism and invited him to discuss his work. Over a three-day period in the Ramapo Mountains of New York I came to know George's innovative and individualistic approach to science; his keen mind, his wit and gregarious yet unimposing nature.

He was then working at the Brookhaven National Laboratories to which he had moved in 1953 and where laboratory and clinical facilities were made available to him. Gathering a substantial team of investigators around him he began expanding his investigative program with particular interest in the biochemical mechanism underlying extrapyramidal and behavioural disorders. Stimulated by his previous work with manganese, as well as the growing body of information concerning the possible role of monoamines in melanin pigment formation, he was deeply involved in defining its role in Parkinson's disease. Though many were exploring the therapeutic value of monoamines particularly dopa in the treatment of parkinsonism, none approached it with the tenacity and courage of George Cotzias. Where others were constrained by adhering to the usual principles of therapeutics, he felt that the brain mechanisms were unique and required a more adventurous approach. The dramatic effects of reversing the symptoms of parkinsonism by giving large doses of L-dopa which were achieved by the Cotzias's group is undoubtedly one of the major milestones in the treatment of neurological disorders. Not only have literally millions with the disease benefited from its use, but the therapeutic principle has found applicability to other disorders. Despite being hailed by some as a panacea for Parkinsonism, Cotzias was the first to admit that it offered only a prolonged holiday from the symptoms and much more had to be done to effect a cure. Indeed, he devoted the last 10 years of his life to looking for more effective means of overcoming the dopamine deficiency in the brain of parkinsonians.

Dr Cotzias received many honours during his life, among which were the Albert Lasker Award in Clinical Medical Research in 1969, the Borden Award in 1972 and the Oscar B. Hunter Award from the American Society of Clinical Pharmacology and Therapeutics in 1973. His native country recognised his contributions with an Honorary Doctor of Medicine degree from the National and Kapodistrian University in Athens, and in

appointing him Grand Commander of the Royal Greek Order of the Phoenix and the Archon Actuarius of the Ecumenical Orthodox Patriarchate.

At the time of his death he was Professor of Neurology at the Cornell Medical College, Attending Physician at New York Hospital and a Special Assistant to the President of the Memorial Sloan-Kettering Cancer Center. He had accepted these positions in 1974, moving his laboratories and personnel just prior to becoming ill. Despite this, he continued to be active and involved in a number of new fields of investigation related to the aging process in the nervous system and mechanisms involved in carcinogenesis. Undoubtedly, this work will be carried forward by his colleagues and collaborators, many trained by him over the years. Yet his presence will be sorely missed in the scientific community throughout the world.

Melvin D. Yahr

C. F. Hickling

DR C. F. HICKLING, CMG, ScD, who died on 14 June at the age of 74 had a unique influence on the development of marine, lake and river fisheries and fish culture over the wide extent of the former British Empire. He was educated at Taunton School and then at St. Catherine's College, Cambridge, to join, in 1927, the staff of the Fisheries Laboratory at Lowestoft. He was a born fisheries naturalist, as keenly interested in the methods of fishing and in the fishermen as in collecting data and interpreting their significance. His prime concern was with the hake fishery, to rise and then fall during this period. It has rightly been said that our knowledge of the biology of that fish owes everything to him. This formed the subject matter of a series of reports largely summarised in his Buckland lectures for 1934 on *The Hake and the Hake Fishery*.

During the war he was Port Fishery Officer at Milford Haven. In 1945 he was appointed Fisheries Adviser to the Secretary of State for the Colonies. In 1961, a year before his retirement, he was transferred to the Department of Technical Co-operation. His activities were literally world-wide, on innumerable islands in the Caribbean and Pacific, in East and West Africa, in Malaya, Singapore, Hong Kong and Sarawak. He advised on river fisheries in Nigeria, on those of the great lakes of Central Africa—then on those of

the created Lake Kariba—and promoted development of sea fisheries off African and Pacific coasts.

He was responsible for the organisation of training courses for fisheries officers appointed during this belated period of colonial activity, also for advice on the location and planning of research laboratories notably at Entebbe in Uganda, in Zanzibar, on Lake Nyasa, at Freetown, Sierra Leone and at Singapore. The largest scheme, carried through during the communist troubles, was the Tropical Fish Culture Institute at Malacca of which, there happily accompanied by his wife, Marjory, he was Acting Director in 1957-59. There he succeeded in crossing strains of *Tilapia mossambica* to produce unisexual cultures which grew without the runting that accompanies the early reproductive activities in these fish.

It was a notable experience, as the writer can testify, to accompany Fred on a tour. He moved from the Governor's residence to the humblest fish landing, advising on scientific and commercial policy and then on the fitting of outboard motors to dug-out canoes, on the quality and handling of nets and on preserving and marketing of the catch. He was rightly described as a 'one man FAO'.

He leaves much of his wide knowledge to posterity in the pages of his books on *Tropical Inland Fisheries* and *Fish Culture*. All who knew him remember him with deep affection, so utterly the right man in the right place. We think also of his widow and two sons.

Maurice Yonge

J. A. Robbie

JAMES ANDREW ROBBIE, FRSE, FGS, died in Edinburgh in his 67th year on 19 May 1977. Born near Laurencekirk he graduated BSc with Honours in Geology at Aberdeen University in 1934. After a short period as Demonstrator in the Geology Department there he was appointed Geologist in the Geological Survey of Great Britain in 1935. He served initially in southern England carrying out geological surveys in the vicinity of Chatham, Bridport and Yeovil. Publication of his work was delayed by the Second World War but eventually appeared as a paper on the Chalk Rock at Winterborne Abbas in his favourite county of Dorset and contributions to the Geological Survey Memoirs on Chatham and Bridport and Yeovil. Although concerned with water

supply, mineral resources and airfield construction during the war he was located in London, a period of his life which evoked vivid tales of the blitz and food shortages, the latter being the greater burden for the gourmet.

In 1947 he was transferred to Belfast as part of the team which initiated the Geological Survey of Northern Ireland. On promotion to District Geologist in charge of the Belfast Office in 1959 he was instrumental in establishing the close relationships with central and local government which have resulted in a key role for the Geological Survey in the area. The acme of his geological career came in Northern Ireland with many studies including those of the Mourne Mountain granite as revealed in the Slieve Binnian Tunnel and of the Carboniferous stratigraphy proved by boreholes between Coalisland and Dungannon, published in the Bulletin of the Geological Survey of Great Britain. The scope of his experience and knowledge is demonstrated by his accounts of lithology, stratigraphy and structure of strata of the Dalradian, Silurian, Carboniferous, Triassic, Rhaetic, Liassic, Cretaceous, Tertiary and Pleistocene in memoirs describing the geology of the Dungannon, Ballycastle and Belfast districts.

When promoted to Edinburgh to be Assistant Director in charge of all the activities of the Institute of Geological Sciences in Scotland he was so deeply entrenched in Northern Ireland geology that his model became something of an anathema to the Scots in their analyses of local geology. While involved in the expansion of IGS activities both on land and offshore he played a major role in planning and developing Murchison House, the new headquarters of IGS in Scotland, which was nearing completion as he retired in 1975.

Elected a Fellow of the Royal Society of Edinburgh in 1968 he was appointed Vice President in 1975. He was a Fellow of the Geological Society of London, served as President of the Edinburgh Geological Society from 1971-73, and was elected an Honorary Member of the Belfast Geologists' Society of which he was President twice. In his official capacity he served on the Council of Management of the Macaulay Institute for Soil Research from 1969 until 1975.

A quiet, shy, self-effacing man Jim Robbie shunned the social scene and yet enjoyed nothing more than to meet friends over a glass of his favourite national spirit. He faced his retirement full of plans for remodelling his garden, refurbishing his beloved old car and travelling the world, plans only in the earliest stages of fulfillment when he died.

G. I. Lumsden